The neural basis of epilepsy and cognitive impairment in children born preterm
– A neuroimaging study –

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Thesis submitted to the University of London in partial fulfilment for the degree of Ph.D.

June, 2008

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I, Brigitte Vollmer, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Abstract

The work described in this thesis aimed to investigate the characteristics and neural correlates of epilepsy and cognitive impairment in preterm children.

From a large cohort of preterm children (born at gestational age <33 weeks), those who had developed epilepsy by age eight years were included in the study. Since the main hypothesis was that in those children with epilepsy and/or cognitive impairment additional undetected grey matter abnormalities are present, preterm control children without epilepsy from the same cohort were selected such that balance was achieved with respect to white matter abnormalities identified on neonatal cranial ultrasound.

Conventional structural magnetic resonance (MR) imaging data were analysed qualitatively (visual inspection) for white and grey matter abnormalities. In addition, a quantitative MR imaging analysis method, voxel-based morphometry (VBM), was used for detection of more subtle grey matter abnormalities that may not be detected by purely visual analysis of MR images. Perinatal and neonatal data, data from neurological and psychometric assessments, from the medical history and data obtained from electroencephalography (EEG) were analysed and related to neuroimaging findings.

Visual analysis of MR images identified brain abnormalities that had gone undetected on neonatal ultrasound. VBM analysis identified subtle grey matter abnormalities that had not been detected on visual analysis of MR images. VBM-detected grey matter abnormalities were associated with periventricular white matter reduction identified on visual inspection of MR images. The analyses provide evidence for epilepsy and/or cognitive impairment to be related to both reduction of periventricular white matter and subtle VBM-detected grey matter abnormalities. The data suggest that using combined information from visual inspection of MR images and VBM analysis of grey matter improves significantly the prediction of epilepsy and cognitive outcome compared to using findings from clinical variables alone. The data also indicate that in this study group, the extent of brain injury had a stronger effect on cognitive outcome than the presence of epilepsy.

Contents

List of tables

List of figures

List of appendices

Acknowledgements

First, I would like to thank my supervisors, Professor Brian Neville, Professor Alan Connelly and Professor John Wyatt, for giving me the opportunity to undertake this research and for their supervision. I am extremely grateful to Professor David Gadian who, following Professor Connelly's move to Australia, took over supervision from Professor Connelly in the later stages of the writing of this thesis. I am very grateful to my supervisors for their support and guidance throughout this work. In particular, I am very thankful for their excellent and continued teaching, many on-the-spot discussions of the data, whilst furthering my independence and scientific thinking.

Professor Brian Neville has been extremely supportive during all the time we have been working together and I have been very fortunate and am very grateful to have been given the opportunity to work clinically and conduct research under his lead. Professor Alan Connelly was extremely patient in teaching me about MR physics and the application of MR imaging methods to investigation of brain structure following early brain injury; over the years, he was always available for discussion of my never ending questions, not only regarding MR imaging, but also about research and science in a wider sense. Professor John Wyatt introduced me to research and clinical work in the field of neonatal neurology, and I would particularly like to thank him for that, as well as for many valuable discussions, not only about research and clinical issues, but also about ethics connected to the field of neonatology and the long term consequences of preterm birth.

I am enormously grateful to Dr Martin King who, in addition to devoting considerable thought, effort and time supervising the statistical analyses for this study, never became tired of answering my questions about statistics and study design. I am also very grateful to Dr Elizabeth Isaacs who was always available for discussion, gave valuable advice regarding the analyses that included data from psychometric assessments and has never stopped encouraging me to continue with this work.

I would like to thank Dr Stewart Boyd, Consultant Neurophysiologist, and Dr Kling Chong, Consultant Paediatric Neuroradiologist, both of whom have been very generous with sharing their expertise, dedicating time and giving advice regarding the EEG data and the neuroimaging data.

I am very grateful to the children and their families who agreed to participate in this study, to Jan Townsend for all the help in recruiting the children for this study and to Cheryl Richardson and Clare Marshall for scanning the children.

During my time at the Neurosciences Unit and the Institute of Child Health, I have been fortunate to work with fellow PhD students and colleagues who not only were always prepared to discuss research, to give advice and constant support, but also have become friends, and I would like to mention particularly Dr Gillian Craig, Dr Caroline Edmonds, Dr Richard Chin and Dr Rod Scott.

Last but not least, I would like to express my gratitude to the late Dr Ann Stewart, who, in the very early stages of me venturing out into the complex field of long term follow-up of preterm children, took me under her wing, very generously shared her extensive knowledge and taught me skills that are essential for working in this field.

Without continuing support from my parents, my sister, my friends Gudrun Schmitt, Brian Richardson and Elizabeth Isaacs, it would not have been possible to complete this thesis.

Part I

Introduction, hypotheses and structure of thesis, background and methods

This part of the thesis gives a short introduction (chapter 1) followed by presentation of the hypotheses and description of the further structure of the thesis (chapter 2). Chapter 3 gives an overview on the relevant background and chapter 4 presents the methods that have been chosen for investigation of the research questions. Chapter 4 also includes a detailed description of the study population.

Chapter 1: Introduction

Despite substantial advances in perinatal and neonatal care over the last decades, infants born very preterm (<32 weeks of gestation) remain at high risk of developing long term neurodevelopmental impairments. About 10-15% of these infants will develop major neurodevelopmental impairments such as severe neuromotor impairment (cerebral palsy), global cognitive delay, severe visual impairment, and epilepsy. Of the remainder, about 20->30% are at risk for specific cognitive impairments, behavioural problems and/or minor neurological signs (for review see Hack and Fanaroff, 2000; Bhutta et al, 2002).

Although not very frequent, epilepsy is an important component of the neurological impairments that occur in preterm born children. The prevalence of epilepsy in preterm infants is clearly higher than in the normal population and has great impact on the everyday life of these children and their families, especially when co-existing with cognitive impairment, neuromotor impairment and behavioural problems.

Historically, the main neurological syndrome following preterm birth, particularly before the survival of very preterm infants, was "spastic diplegia" in which overall cognitive impairment was absent or minimal, and epilepsy very uncommon. However, closer investigation of this population has revealed a subgroup with both epilepsy and cognitive impairment, which requires further explanation in terms of the pathological basis. It has been shown that the typical brain lesion in the preterm infant is damage to white matter and it made therefore logical but perhaps simplistic sense to regard the impairments following preterm birth as white matter disorders. However, the presence of epilepsy and cognitive impairment strongly suggest additional grey matter damage. This has not previously been investigated in detail, partly because tools for thorough and detailed investigation of this question have only become available recently.

Magnetic resonance imaging (MRI) provides a powerful tool to investigate brain structure and function. The high spatial resolution, whole brain coverage and wide range of tissue

contrast available provide improved lesion detection compared to cranial ultrasound. In addition to purely visual analysis of MR images, statistically based methods of image analysis have recently been developed that allow detailed investigation of brain structure and increased sensitivity in detection of subtle abnormalities in brain structure following injury to the immature brain. One such method is voxel-based morphometry (VBM), which is the quantitative image analysis method that has been used in the present work.

The work presented in this thesis aims to investigate the characteristics and neural correlates of epilepsy and overall cognitive function in a clearly defined population of preterm children using clinical data and visual analysis of MRI data as well as a statistically based image analysis method (voxel-based morphometry, VBM) for detection of subtle brain abnormalities.

Chapter 2: Hypotheses and structure of thesis

The work described in this thesis focuses on the investigation of relationships between alteration of brain structure and epilepsy and/or cognitive impairment in very preterm children. In particular, it aims to test the hypothesis that in preterm children, epilepsy and/or cognitive impairment are associated with grey matter abnormalities that are additional to the white matter lesions identified by neonatal cranial ultrasound.

This work focuses mainly on the investigation of relationships between magnetic resonance imaging findings (from both visual analysis and analysis of the MR data by voxel-based morphometry, VBM) and the manifestation of epilepsy and cognitive impairment. It also includes analysis of perinatal and neonatal data, data obtained from neurological, neuropsychological and neurophysiological assessments. In addition, the application of VBM in a paediatric population and in patients with visible brain lesions is investigated.

The main hypothesis is tested by addressing the following questions:

- Is there an association between epilepsy and (a) white matter or grey matter abnormalities identified on visual inspection of MRI; (b) grey matter abnormalities identified by voxel-based morphometry?
- Is there an association between cognitive impairment and (a) epilepsy; (b) white matter abnormalities or grey matter abnormalities identified on visual inspection of MRI; (c) grey matter abnormalities identified by voxel-based morphometry?

In addition, perinatal and neonatal data will be examined to investigate the following questions:

- Is there an association (independent of the imaging findings) between particular perinatal or neonatal characteristics and subsequent manifestation of epilepsy?
- Is there an association (independent of the imaging findings) between perinatal or neonatal characteristics and overall cognitive outcome?

This thesis is divided into five parts. Part 1 (chapter 1-4) includes the introduction, states the hypotheses and research questions to be investigated. This part also gives an overview on the relevant background for this work, including aetiology and pathology of brain lesions that occur typically in preterm infants. This is followed by a discussion of consequences for brain development and neurodevelopment after an early lesion to the brain, and by an outline of relevant aspects of neuroimaging in the premature newborn, infant and child. The last chapter of part 1 (chapter 4) describes the study population and the methodology used in this study.

Part 2 is divided into two chapters. Chapter 5 deals with clinical characteristics with regard to epilepsy and clinical data such as perinatal and neonatal data, data obtained from neurological examination at term age and at the time of this study and their associations with the manifestation of epilepsy. Chapter 6 presents data on overall cognitive function and associations with clinical data.

Part 3 of this thesis focuses on neuroimaging data, in particular on identification of structural brain abnormalities in the study population. Chapter 7 includes presentation and discussion of findings obtained from visual analysis of MRI images. Chapter 8 first gives some theoretical background on voxel-based morphometry (VBM) and describes the VBM methodology used in this study. This is followed by presentation and discussion of the findings obtained from the VBM analyses of grey matter.

Part 4 consists of two chapters and focuses on associations of structural brain abnormalities identified by neuroimaging with epilepsy and with overall cognitive function. Chapter 9 presents results from analyses that investigate associations between structural brain abnormalities and manifestation of epilepsy and different aspects of epilepsy. Chapter 10 presents the results from analyses that investigate associations between overall cognitive function and neuroimaging findings.

Part 5 (chapter 11) of the thesis includes a general discussion, conclusions drawn from the work and an outline of directions for future work.

Chapter 3: Background

This chapter aims to provide an overview on mortality and morbidity in very preterm infants, with emphasis on neurodevelopmental outcome. The nature and neuropathological consequences of perinatally acquired brain lesions typically seen in preterm infants are discussed. Clinical consequences of injury to the very immature brain are described with special attention to epilepsy and cognitive function. This is followed by an overview on imaging methods that are commonly used for investigation of brain structure – function relationships in preterm infants and children.

3.1 Mortality and morbidity in preterm infants

3.1.1 Mortality

Chances for survival of very low birth weight infants (<1500 grams), especially for extremely low birth weight (<1000 grams) and extremely low gestational age (<28 weeks) infants, have improved in the last decades. In a review of the literature on infants born before 26 weeks of gestation weighing less than 800g in the 1990s, Hack and Fanaroff (2000) reported that survival rates at 23 weeks` gestation range from 2% to 35%, at 24 weeks gestation 17% to 62% and at 25 weeks` gestation are as high as 72%. There are still wide variations that may be accounted for by different regional criteria for starting and withdrawing treatment, differences in care and different entry criteria in follow-up studies. Increase in survival rates, especially for the most immature babies, is usually attributed to advances in perinatal and neonatal care such as the introduction of surfactant (Jobe, 1993) and the use of antenatal steroids (Gardener, Papile and Wright, 1995; Wright et al, 1995; Volpe, 2001).

3.1.2 Morbidity

Major neonatal morbidities increase with decreasing birth weight and decreasing gestational age (Cooke, 1996; Costeloe et al, 2000; Saigal and Doyle, 2008). In surviving babies born at 24-25 weeks of gestation, the majority are reported to have at least one neonatal complication (Fanaroff et al, 2007). Important morbidities in the neonatal period are chronic lung disease, necrotizing enterocolitis, severe infections, retinopathy of prematurity and, most importantly for later neurodevelopmental outcome, perinatal brain injury. Hormonal factors, such as neonatal transient hypothyroxinaemia (Reuss et al, 1996; Kok, Briet and van Wassenaer, 2001), and nutrition (Lucas and Morely, 1998) have recently been recognised as additional factors that are likely to influence later outcome.

Although mortality in preterm infants has decreased, long term outcome is still a cause for concern. Poor growth in terms of weight, height and head circumference is common in infants born preterm, as is chronic lung disease (Hack and Fanaroff, 2000; Greenough, 2000). However, this present study focuses on long-term neurodevelopmental outcome and therefore a detailed discussion of general childhood morbidities occurring in preterm infants is beyond the scope of this thesis.

There are numerous studies that report that overall rates of neurodevelopmental impairments and disabilities have mainly remained unchanged when children born in the 1990s are compared with children born before the 1990s (for review see Hack and Fanaroff, 2000). Of the children born before 32 weeks of gestation, about 10-15% will develop major neurodevelopmental impairments including severe neuromotor impairments, global cognitive delay, severe impairment of visual function, sensory hearing loss and epilepsy (Roth et al, 1993, 1994; Amess et al, 1998; Hack and Fanaroff, 1999, 2000). Of the remainder about 20-30% will develop "minor" neurodevelopmental problems including specific cognitive deficits (e.g. in visual processing, mathematical skills, memory function), behavioural problems (e.g. attention deficit), often in conjunction with minor neurological signs (Saigal et al, 1991; Jongmans et al, 1996; Botting et al, 1998; Krägeloh-Mann et al,

1999; Bhutta et al, 2002; Aylward, 2002). A more detailed discussion of neurodevelopmental consequences of prematurity is given in section 3.3 of this chapter.

3.2 Brain lesions in the preterm infant

Preterm infants as immature as 23 weeks of gestation age are now frequently viable. At this stage of brain development many aspects of neuronal organisation, such as synaptic formation, dendritic growth, glial proliferation and differentiation have still to be established and myelination is only about to start. Thus, the brain at such an early developmental stage is very susceptible to a variety of injurious stimuli.

In the preterm infant, the characteristic brain lesions are of hypoxic-ischaemic and/or haemorrhagic origin, and are typically located in the periventricular white matter (Friede, 1989; Paneth et al, 1994). There is now increasing evidence that intrauterine infection/inflammation might, in addition to the inflammatory reaction following ischaemia, have a direct toxic effect on immature oligodendroglia (for review see Rezaie and Dean, 2002). Therefore, I will expand the term "hypoxic-ischaemic" injury and will be using the term hypoxic-ischaemic/inflammatory injury in the following.

Although aetiology, and usually imaging findings as well (see e.g. DeVries et al, 1998), are distinct for hypoxic-ischaemic/inflammatory and haemorrhagic injury, it has been suggested that it is not always possible to distinguish between these two types of injury to the periventricular white matter (Kuban and Teele, 1984; Kuban, 1998; Kuban et al, 2001). For example, Kuban et al (2001) reported on the topography (established by neonatal cranial ultrasound) of white matter lesions in a large number of preterm infants and concluded that it was not always possible to clearly designate infants with white matter injury as having periventricular haemorrhagic infarction or periventricular leukomalacia only, and that the term white matter disease of prematurity should be used instead of periventricular leukomalacia or periventricular haemorrhagic infarction when referring to abnormalities defined by ultrasound.

Furthermore, when looking at the significance of lesions for neurodevelopmental outcome in preterm infants, rather than distinguishing between haemorrhagic and hypoxic-ischaemic/inflammatory lesions, there is an alternative approach by categorising lesions as either parenchymal or non-parenchymal, regardless of aetiology. A possible categorisation would then be: a) periventricular white matter damage (including periventricular leukomalacia, haemorrhage that affects periventricular tissue and probably ventricular dilatation since there is evidence that ventricular dilatation with or without preceding haemorrhage reflects some degree of white matter damage (see e.g. Leviton and Gilles, 1996; Paneth, 1999)), and b) intraventricular haemorrhage without involvement of periventricular brain tissue.

In addition to white matter injury neuropathological studies (e.g. Friede, 1989; Paneth et al, 1994) indicate that there is also injury to basal ganglia, selective neuronal injury in the basis pontis and the subiculum of the hippocampus (pontosubicular necrosis), the cortex and the cerebellum. Frequently, these lesions are seen in association with periventricular white matter lesions of mainly ischaemic origin (Friede, 1975; Takashima, 1982). However, the significance of these grey matter lesions on long term outcome is as yet unclear.

3.2.1 Hypoxic-ischaemic/inflammatory brain lesions - periventricular leukomalacia

Hypoxic-ischaemic/inflammatory brain injury in preterm babies leads to periventricular leukomalacia (PVL), which can occur up until term (Volpe, 2001). The risk for PVL is high between 23-32 weeks postconceptional age (Back et al, 2001). The pathogenesis of PVL is still not fully understood. However, the current view is that PVL most likely results from a combination of the immature vasculature of the brain and impaired perfusion and/or hypoxia during a period of increased vulnerability. In addition, there is increasing evidence from epidemiological and experimental studies that intrauterine infection/inflammation and chorioamnionitis are contributing factors that might, in addition to the inflammatory

reaction following ischaemia, have a direct toxic effect on immature oligodendroglia (Dammann, 1997; Kinney, 1998; Rezaie and Dean, 2002).

PVL is frequently associated with secondary haemorrhage within the area of leukomalacia and this may co-exist with intraventricular/periventricular haemorrhage (Friede, 1989; Paneth et al, 1994; Volpe, 2001).

3.2.1.1 Neuropathology - regional and cellular aspects

In 1962, Banker and Larroche (Banker and Larroche, 1962) described the neuropathological characteristics of PVL with regards to topography and evolution of the lesions. They also suggested an association to the vascular border zones. In their study more than half of the babies with periventricular infarcts were babies born preterm. There are very early neuropathological reports by Parrot (1868, 1873) who described periventricular infarcts (Parrot, 1868) and attributed them to malnutrition. In 1873, Parrot (Parrot, 1873) gave a very detailed description of the lesions as "chalky plaques" located some millimeters from the ependymal surface of the lateral corners of the ventricles, sparing grey matter. He also described softening of the plaques, forming cavities filled with fluid that did not communicate with the ventricles. He noted that these cavities were almost exclusively seen in the immediate periventricular tissue. Some years later, Schwartz (Schwartz, 1924, 1927) re-described periventricular infarcts. However, the study by Banker and Larroche (1962) is the most complete with regards to providing information about clinico-pathological relationships.

The neuropathological features of PVL consist of both focal periventricular necrosis and more diffuse, widespread white matter injury (Leviton and Gilles, 1996; Kinney, 1998). Diffuse injury can occur independently of overt deep focal lesions (Volpe, 2001). There now seems to be increasing evidence, including from neuroimaging studies using diffusion weighted MRI in preterm infants at term, that diffuse injury is likely to be the principle

feature of white matter injury in the preterm infant (e.g. Maalouf et al, 1999, 2001; Inder et al, 2003; Counsell et al, 2003).

Deguchi et al (1999) performed neuropathological studies on brains of infants with PVL born between 22–41 weeks of gestation and defined the lesions histochemically as focal (single or multiple foci in deep white matter around lateral ventricles), widespread (deep white matter without involvement of subcortical areas) and diffuse (periventricular to subcortical areas). Their findings suggest that there is an association between the type of PVL and gestational age. The younger infants tended to have widespread lesions whereas the more mature infants more often had focal lesions.

Focal necrosis typically is bilateral and located anteriorly to the frontal horns of the lateral ventricles, the lateral corners of the lateral ventricles and the lateral surfaces of the occipital horns of the lateral ventricles. Less frequently involved is the area around the temporal horns, and usually the lesions are separated from the ventricles by a small layer of glial tissue (Friede, 1989). There is evidence that in focal PVL, a few hours after a hypoxic-ischaemic insult the lesion resembles the acute appearance of focal infarction, affecting all cellular elements. Coagulation necrosis occurs at the site of the focal lesion and neuroaxonal swelling is present. There is also some evidence for axonal rupture and subsequent glutamate release into periventricular white matter (Volpe, 2001). The subsequent cellular responses (e.g. microglia infiltration, astrocyte proliferation, endothelial hypoplasia, appearance of foamy macrophages) lead to tissue destruction, and cystic cavities develop within one to three weeks after the injury.

The neuropathology of the diffuse component of PVL has been emphasised by Gilles and co-workers, particularly in the report by Gilles and Murphy (1969), which described the results of an autopsy study of 200 brains of infants and viewed the glial reactions as key factors of white matter damage. (They referred to this type of injury as "perinatal telencephalic leucoencephalopathy"). As already mentioned above, the more diffuse type of injury may occur with or without focal necrosis and, in contrast to the regions with focal necrosis, rarely undergoes cystic changes. In contrast to the focal type of injury, the more

diffuse component of the injury does not affect all cellular elements. There is evidence from neuropathological studies that it is mainly a cell specific lesion affecting oligodendrocyte precursor cells (pre-oligodendrocytes) or early differentiating oligodendrocytes, which subsequently leads to impairment of myelination (Gilles and Murphy, 1969; Barth, 1980; Paneth, 1990; Ozawa et al, 1994; Takashima, 1995; Haynes et al, 2003). Interestingly, Haynes et al (2003), in an autopsy study on 17 infants with PVL, reported that the abnormalities were strictly confined to white matter and that cortical grey matter seemed to be spared. This may indicate that reduced cortical grey matter volume in preterm infants with PVL as indicated in an MRI study (Inder et al, 1999) of preterm infants at term (a proportion of whom had diffuse PVL) is likely to be a secondary phenomenon and not related to a primary injury to cortical grey matter.

3.2.1.2 Pathogenesis

The pathogenesis of PVL is likely to be related to three major interacting factors that are characteristic of the immature brain, and probably lead to ischaemia in the periventricular white matter and subsequent injury (Volpe, 2001; Hagberg, Peebles and Mallard, 2002). First, periventricular vascular anatomical factors (vascular border and end zones; Takashima and Tanaka, 1978; Rorke, 1992), and second, pressure passive cerebral circulation (with danger of systemic hypotension; Pryds, 1991) combined with the intrinsic vulnerability of the white matter in the immature brain (such as limited vasodilatory capacity, relatively active anaerobic glycolysis, vulnerability of differentiating glial cells to free radicals). The third major factor is likely to consist of toxicity of extracellular glutamate to oligodendroglia, possibly following the disruption of axons after hypoxia-ischaemia (for detailed review see Volpe, 2001). As mentioned in previous sections, there is now increasing evidence that in addition to ischaemia-reperfusion, intrauterine infection/inflammation with cytokine release and possibly direct toxic effects of bacterial endotoxins are likely to be important factors (Duggan, 2001; Hagberg, Peebles and Mallard, 2002; Hagberg and Mallard, 2005).

3.2.1.3 Long term neuropathological consequences of hypoxic-ischaemic/inflammatory injury

The neuropathological long term consequences of hypoxic-ischaemic/inflammatory injury depend on the size of the initial lesion and partly on whether the lesion is primarily of focal or diffuse nature. Neuropathological features include the development of cystic cavities that constrict with progression of gliosis, of irregular ventricular margins and ventricular dilatation. Furthermore, the injury leads to reduced white matter in the periventricular area, probably reflecting impairment of myelination following injury to early differentiating oligdendroglia/precursors (Volpe, 2001). On a microscopic level oligodendroglial deficiency is the dominant finding in both focal and diffuse PVL.

3.2.2 Haemorrhagic lesions – germinal matrix haemorrhage, intraventricular haemorrhage, and haemorrhagic parenchymal infarction

Haemorrhagic lesions mainly occur in the most immature infants and there is a positive correlation between the incidence of haemorrhage and decreasing gestational age (Perlman and Volpe, 1986; deVries, 1988). Haemorrhage usually occurs within the first 72 hours of life and very rarely after the first week of life (Volpe, 2001).

3.2.2.1 Neuropathology – regional and cellular aspects

Haemorrhagic lesions are mainly unilateral, and in the bilateral cases almost invariably asymmetrical (Volpe, 2001). In the majority of cases, haemorrhage starts in the subependymal germinal matrix, which is located ventrolateral to the lateral ventricles (Hambleton and Wigglesworth, 1976; Pape and Wigglesworth, 1979) and contains a rich capillary bed supplied by Heubner's artery (a branch of the anterior cerebral artery) draining directly into the terminal vein. However, much of the vasculature of the germinal

matrix seems to consist of an immature net of vessels within which arterioles, venules and capillaries are difficult to distinguish. The microvasculature of the germinal matrix has been described as a network of capillaries and sinusoids. These vessels are not supported by muscular coats (Kuban and Gilles, 1985) and are therefore very fragile. At the end of the second trimester the germinal matrix is one of the most metabolically active regions of the brain and receives a high proportion of cerebral blood flow.

The germinal matrix serves as source for cerebral neuronal precursors between 10 to 20 weeks of gestation and, in the third trimester, provides glial precursor cells that develop into oligodendroglia and astrocytes. It then gradually decreases in size until nearly complete involution at about 36 weeks of gestation. Between 28 and 32 weeks of gestation it is most prominent in the thalamo-striate groove at the level of the head of the caudate, slightly posterior to the foramen of Monro, a site that is the most common location of germinal matrix haemorrhage (GMH) in preterm infants of 29 weeks of gestation or more (Hambleton and Wigglesworth, 1976; Paneth et al, 1994). There is some evidence from neuropathological studies that before 29 weeks of gestation haemorrhage most frequently develops in the matrix over the body of the caudate (Hambleton and Wigglesworth, 1976).

The exact source of bleeding is still debated. Most of the early neuropathological studies came to the conclusion that GMH was of venous origin (Patten and Albers, 1933; Gruenwald, 1951). Towbin (1968) and Larroche (1964) argued that GMH is preceded by infarction of the germinal matrix with subsequent stasis and weakening of the terminal vein wall. Until the work of Hambleton and Wigglesworth (1976) and Pape and Wigglesworth (1979), the view that GMH was of venous origin was unchallenged. In animal studies they showed by injections in the carotid artery that the injected material mainly leaked into the capillary bed supplied via Heubner`s artery. In contrast, injection in the jugular veins caused ruptures at the junction of the deep and cortical venous system but not of the terminal vein. More recently, Volpe (1989) as well as Lou, Lassen and Friis-Hansen (1979) suggested a variant of the view expressed by both Towbin (1968) and Larroche (1964). They suggested that GMH haemorrhage may be a form of reperfusion injury. According to this view hypoperfusion of the germinal matrix weakens the vessel walls through ischaemic

injury and, if followed by re/hyperperfusion, leads to haemorrhage. It should be noted that the main weakness of Towbin's and Larroche's view (although supported by some animal studies) is the absence of infarction in germinal matrix tissue at autopsy of human brains with evidence of subependymal haemorrhage (Leech and Kohen, 1974; Hambleton and Wigglesworth, 1976). In many cases, the ependymal layer that separates the germinal matrix from the lateral ventricles ruptures, blood enters the lateral ventricles and spreads throughout the ventricular system, which leads to intraventricular haemorrhage (IVH). There is an association of GMH/IVH with a characteristic parenchymal lesion, a region of haemorrhagic necrosis in the white matter surrounding the lateral ventricles, located mainly dorsally and laterally of the external angle of the ventricle (haemorrhagic parenchymal infarction). Neuropathological studies in both animals (Takashima, Ando and Takeshita, 1986) and humans (Gould et al, 1987) suggest that this lesion is not caused by extension of the IVH but by venous infarction (most likely following an obstruction of terminal veins by GMH/IVH) with secondary haemorrhage into the previously infarcted area. There seems to be some overlap in the pathogenesis of PVL and haemorrhagic parenchymal infarction, and therefore it is not surprising that these lesions can coexist (Volpe, 2001; Larroche, 1964).

In GMH/IVH and haemorrhagic parenchymal infarction, there are distinct pathological features on a cellular level: In very early stages of GMH/IVH, erythrocytes are seen extravasally and the matrix is still without edema, thrombosis, necrosis, or ischaemic changes. On extension of the haemorrhage, changes in the normal cellular architecture occur. The first changes in the germinal matrix include the development of oedema, glial nuclear vesiculation and pyknosis. Then germinal matrix cells disappear and macrophages become increasingly present. Subsequently, the germinal matrix collapses and becomes clustered with haemosiderin-laden macrophages. A few weeks after the haemorrhage the germinal matrix is either replaced by a cyst or reactive astrogliosis occurs (Paneth, 1994). An important consequence of these changes is the destruction of glial precursor cells (see below). The haemorrhagic component following an infarction consists of perivascular haemorrhages that closely follow the distribution of the medullary veins in periventricular white matter (Gould et al, 1987; Friede, 1989).

3.2.2.2 Pathogenesis

The pathogenesis of GMH/IVH is likely to be multifactorial and it is probably a combination of intravascular factors (increase in cerebral blood flow, decrease followed by re-perfusion, increase in venous pressure, platelet and coagulation abnormalities), vascular factors (vulnerability of matrix vessels, tenuous integrity of capillaries) and extravascular factors (deficient vascular support for capillaries, fibrinolytic activity) that lead to haemorrhage (for a detailed review see Volpe, 2001). Not all the factors need to be present, and different combinations of these factors may be operating in different patients. As described above, the pathogenesis of haemorrhagic parenchymal infarction appears to be causally related to GMH/IVH.

3.2.2.3 Long-term neuropathological consequences of haemorrhagic injury

As outlined above, GMH/IVH leads to the destruction of the germinal matrix with its glial precursor cells. The potential effect of these neuropathological changes on brain development is discussed in more detail in the following. If the haemorrhage is extensive, posthaemorrhagic hydrocephalus may develop by spreading of blood from one or both lateral ventricles to the third and fourth ventricle with subsequent tamponade of the ventricular system (Friede, 1989). In haemorrhagic periventricular infarction, necrotic changes occur and may in many cases develop into cavitations not unlike purely ischaemic lesions. However, the cavitations following a haemorrhage are smooth-walled cysts with very little gliosis (Friede, 1989), and after about eight weeks these cysts are usually integrated into the dilated ventricle (unlike the cysts of periventricular leukomalacia, which are self-absorbing).

3.2.3 Effects of insults to the immature brain on subsequent brain development

There is increasing evidence that beyond the primary insult to mainly the periventricular white matter, injury to the immature brain has effects on subsequent brain development. A number of neuropathological studies (e.g. Kostovic et al, 1989; Marin-Padilla, 1997; Kostovic and Judas, 2002), and more recently magnetic resonance imaging studies (e.g. Allin et al, 2004; Vangberg et al, 2006; Nosarti et al, 2008), suggest that white matter injury of both types of origin - haemorrhagic and ischaemic/inflammatory - have long term effects on development of both white and grey matter. It seems likely that these subsequent grey and white matter abnormalities following a primary insult to periventricular white matter could have important implications for neurological and neurodevelopmental outcome in preterm infants

3.2.3.1 Impact on white matter organisation and myelination

It has been suggested that a common feature of all forms of white matter damage in the preterm infant is disturbance of oliogodendrocyte precursors and other factors important for myelination (e.g. Takashima, Iida and Deguchi, 1995; Leviton and Gilles, 1997). For example, results of neuropathologial studies suggest that injury to the periventricular white matter causes damage to transneuronal connecting fibres like corticospinal tracts, thalamocortical fibres, optic radiation, superior occipito-frontal and superior longitudinal fasciculus (Okoshi, Ito and Takashima, 2001). In addition, from neuroimaging studies, there is evidence that delayed or insufficient myelination is a common feature in preterm infants who had periventricular white matter injury diagnosed on neonatal cranial ultrasound (e.g. Skranes et al, 1998).

3.2.3.2 "Acquired cortical dysplasia"

Recent studies by Marin-Padilla (1996, 1997) in infants who died following large haemorrhagic white matter injury have shown alterations of the microstructure of the brain in primarily undamaged areas of grey matter overlying the damaged white matter. These post injury alterations of grey matter are characterised by alterations in structural and functional differentiation of neurons, synaptic profiles, fibre distribution, glial elements and vascular structure. It has been postulated that these alterations are secondary to disturbance of input to and output from the cortex by disruption of the respective white matter axons (Okoshi, Ito and Takashima, 2001). These post injury alterations are likely to be ongoing processes, which start after the primary insult and continue to develop over weeks, months or even years, and have been referred to as "acquired cortical dysplasia" (Marin-Padilla, 1997). It has been suggested that these secondary changes may explain some of the neurological impairments following perinatal brain injury (Marin-Padilla, 2000).

3.2.3.3 Subplate neurons

Another cause for alterations of cortical development in the context of brain injury in the preterm infant is likely to be the destruction of subplate neurons (Evrard, 1992; McQuillen et al, 2003), which are abundant in the subcortical white matter. Subplate neurons are critical for cerebral cortical organisation. Gosh and Shatz (1993) have described the potential functions of the subplate neurons. They provide a site for synaptic contacts for axons ascending from the thalamus and other cortical sites; these axons are called "waiting" axons because their final neuronal targets in the cortical plate have not yet arrived or differentiated, and would therefore be likely to undergo degeneration if they did not have the subplate neurons as transient targets. Also, subplate neurons have been shown to serve as a functional link between these axons and their final cortical targets. Cells that are going to develop into subplate neurons are generated in the germinative zones and then migrate to the primitive marginal zone at about seven weeks of gestation, before generation and migration of neurons of the cortical plate. The times for peak development and regression

of the subplate neuron layers in different regions varies, but the active times of the subplate neurons correspond closely to the times of occurrence of preterm haemorrhagic and ischaemic lesions.

3.3 Neurodevelopmental consequences of perinatally acquired brain lesions

From the perspective of prognosis for neurodevelopmental outcome many studies have shown that the lesions that involve the periventricular white matter, namely cystic PVL and periventricular haemorrhagic infarction, carry a poor prognosis. There is a strong association between such lesions and the major neurological impairments as demonstrated by neonatal cranial ultrasound (e.g. Stewart et al, 1983; Roth et al, 1993; Pierrat et al, 2001) and conventional MRI studies in the neonatal period (Roelants-van Rijn et al, 2001; Woodward et al, 2006; Dyet et al, 2006) or later in childhood (e.g. Cioni et al, 1997; Krägeloh-Mann et al, 1999). Lesions that do not involve periventricular tissue, i.e. germinal matrix/intraventricular haemorrhage, usually have less severe neurological and neurodevelopmental consequences as suggested by studies investigating associations between neonatal cranial ultrasound and long term outcome (Stewart et al, 1983; Vollmer et al, 2003). As mentioned earlier in this chapter, it is very likely that the long term neuropathological consequences following an insult to the preterm brain are similar in all forms of periventricular white matter injury. From the perspective of prognosis for overall neurological and neurodevelopmental outcome it therefore seems justified to categorise the lesions into parenchymal and non-parenchymal lesions, irrespective of whether they are of ischaemic/inflammatory or haemorrhagic origin.

Preterm infants are at risk for developing a range of neurological and neurodevelopmental impairments, and the following areas are commonly affected: motor function, cognitive and behavioural function, and visual function. Although not very common, epilepsy in preterm children is more frequent than in the normal population, often co-exists with severe neuromotor impairment and has severe implications for the life of the affected individuals (for detailed discussion see section 3.3.3 in this chapter). In the more severely affected

children these impairments are more often found in combination than in isolation (Hack and Fanaroff, 2000). The more severe impairments, e.g. severe neuromotor impairment (cerebral palsy), severe visual impairment, can usually be related to location and extent of lesions seen on neonatal ultrasound (Stewart et al, 1983; Roth et al, 1993; Amess et al, 1998) and/or MRI in infancy and childhood (Cioni et al, 1997; Krägeloh-Mann et al, 1995, 1999; Jacobson and Dutton, 2000). However, the less severe impairments, which are frequent in those preterms who survive without major neurological impairment, such as specific cognitive deficits and behavioural problems, remain largely unexplained by visual analysis of conventional MR imaging, and even those preterm children without overt lesions on conventional imaging have a higher risk for neurological and neuropsychological problems than term born children (Olsen et al, 1997; Krägeloh-Mann et al, 1999).

In the following section, an overview on the different areas that are commonly affected in the neurodevelopment of preterm children is given and, using examples from the recent literature, associations between the likely underlying pathology as indicated by neuroimaging and outcome in these different areas is discussed.

3.3.1 Neuromotor impairment, severe visual impairment, sensorineural hearing loss

The major neurological manifestation of brain injury in the premature infant are neuromotor impairments. The neuropathological correlates for these impairments are PVL and/or severe grades of haemorrhage (i.e. haemorrhage with involvement of the periventricular white matter). In contrast to infants with an injury that involves periventricular white matter, who have a high risk for severe impairment of motor function, infants with "uncomplicated" haemorrhage (i.e. where no haemorrhagic parenchymal infarction is present and where no posthaemorrhagic hydrocephalus occurs) have a slightly increased risk for neuromotor impairment when compared with infants without haemorrhage (Stewart, et al 1983; Paneth et al, 1994; de Vries et al, 1998). The severe neuromotor impairments can usually be related to lesions seen on conventional neuroimaging such as cranial ultrasound in the neonatal period (for a review see Paneth et

al, 1994, pp 178-185) and MRI later in childhood (Krägeloh-Mann et al, 1995). The major long-term neuromotor consequence of bilateral periventricular lesions is bilateral spastic cerebral palsy (BSCP), with the typical distribution being such that the lower limbs are more affected than the upper limbs. This would be expected in less severe periventricular injury since the fibres for the lower limbs are located very close to the ventricles. In more severe cases, in which the lesion extends more laterally and into the centrum semiovale and the corona radiata, fibres for the upper limbs will be affected as well. Typically, infants with unilateral haemorrhage show spastic hemiparesis or, with bilateral haemorrhage show asymmetrical spastic tetraparesis (Krägeloh-Mann et al, 1995, 1999; Volpe, 2001). In those preterm children who have extensive involvement of the periventricular white matter, CP often exists in combination with impairment of visual function and global impairment of cognitive function.

A large proportion of preterm children without overt brain lesions and without severe neuromotor deficits exhibit so called "minor neurological signs" (Jongmanns et al, 1996), and findings from previous studies suggest that these minor neurological signs are frequently accompanied by mild cognitive impairment and behavioural problems (see e.g. Olsen et al, 1998).

Cerebral visual impairment, i.e. visual impairment caused by abnormalities in the retro-chiasmatic parts of the visual system (oculo-motor abnormalities, retinopathy of prematurity and refractive errors will not be discussed here), is frequent in preterm infants. It is likely to be caused mainly by disruption of fibres in the optic radiation and/or impairment of higher visual pathways (mainly the dorsal stream), and associative areas (Atkinson and Braddick, 2007). Visual impairment is characterised by delayed visual maturation, abnormal visual acuity, visual field defects, and visual-perceptual deficits (Jacobson and Dutton, 2000). In preterm infants with periventricular white matter damage (and motor impairment) several studies have shown a strong association between the severity of visual impairment and severity of white matter lesions on conventional MR imaging (Atkinson et al, 2008), in particular, the degree of periventricular white matter reduction (Lanzi et al, 1998; Krägeloh-Mann et al, 1999; Cioni et al, 2000). It has,

however, also been shown that preterm children who have no major neurological impairments and/or normal findings on structural conventional MRI frequently show deficits in different aspects of visual processing (Luoma et al, 1998; Cooke et al, 2004). A substantial proportion of preterm children have difficulties in visuo-motor skills (see e.g. Jongmanns et al, 1996), and there is some evidence that these problems are more likely to be influenced by deficits in fine motor function than by visual impairment following retinopathy of prematurity (Goyen et al, 2006) or, in those without retinopathy, by impaired visuo-perceptual skills (Aylward, 2002).

Preterm infants are also at risk of sensorineural hearing loss (Hack and Fanaroff, 2000; Marlow et al, 2005) although it appears that this is not very frequently seen without other co-existing impairments.

3.3.2 Cognitive function and behaviour

Preterm infants at pre-school and school age consistently show poorer cognitive performance and increased frequency of behavioural problems compared to term born children (Bhutta et al, 2002; Anderson and Doyle, 2003; Taylor et al, 2006). There is a substantial body of literature showing that lower birth weight and lower gestational age are significantly correlated with decrease in IQ scores (for review see Hack and Fanaroff, 2000; Bhutta et al, 2002).

Most studies conducted over the last decades report global impairment of cognitive function rather than impairment of specific cognitive domains, with average full scale intelligence score (IQ) scores being 8-15 points lower than in term born peers (Saigal et al, 1991; Wolke and Meyer, 1999; Marlow et al, 2005). A high rate of global cognitive impairment is found in combination with CP (with the exception of the CP subtype in which the impairment is limited to the lower limbs), with about a third of preterm infants with CP having a severe impairment of cognitive function. Only recently, studies have been emerging that report deficits in specific cognitive domains such as executive function

(Böhm, Smedler and Forssberg, 2004; Edgin et al, 2008), memory function (Böhm, Smedler and Forssberg, 2004), different aspects of visual processing and behavioural function (Anderson and Doyle, 2003; van de Weijer-Bergsma, Wijnroks and Jongmans, 2008) in preterm children at school age, even in the context of average IQ scores. There is now increasing recognition that follow–up studies need to look beyond IQ measurements in order to understand better the nature and profile of cognitive deficits and the impact on educational performance in this population (Aylward, 2002).

It has been shown that severe impairment of overall cognitive function can, to an extent, be related to the location and severity of periventricular lesions on neonatal ultrasound (Roth et al, 1993; de Vries et al, 1998; Vollmer et al, 2003) and, later in childhood, to lesions detected by visual inspection of conventional MRI. On MR imaging, the structural correlates of severe cognitive impairment include large periventricular parenchymal lesions, bilateral severe white matter reduction, and/or cerebellar atrophy (see e.g. Krägeloh-Mann et al, 1999). From recent MRI studies that measured total and regional brain volumes, there is also evidence that smaller regional brain volumes are linked to poorer cognitive performance in those preterm children without major neuromotor problems (Peterson et al, 2000, 2003).

However, it has been difficult to establish a clear relationship between brain lesions identified on conventional neuroimaging and mild global cognitive impairment (Stewart et al, 1999; Skranes et al, 2008), and/or specific cognitive deficits in the context of normal overall IQ in preterm children (Skranes et al, 2008). It is likely that the underlying anatomical substrates of cognitive and behavioural deficits in preterm infants without major neurological impairments are too subtle to be detectable on visual inspection of conventional MRI.

Recently, new MR image acquisition and analysis techniques have become available, which make it possible to investigate subtle changes in brain structure in more detail, for example, MR diffusion imaging for detailed investigation of white matter. Results of studies investigating the association of structural abnormalities identified by MR diffusion

imaging and associations with cognitive function at school age are only just emerging. Findings from these studies indicate that some of the specific cognitive and behavioural problems encountered by preterm children at school age can be associated to subtle, often widely distributed, white matter abnormalities (Skranes et al, 2007; Constable et al, 2008). Voxel-based morphometry (VBM) is a recently developed statistically based method for the analysis of MR data. Recently, this tool has been used for analysis of 3D T1 weighted MR data sets in unselected groups and groups of neurologically normal preterm children/adolescents for investigation of associations between subtle brain abnormalities and cognitive and/or behavioural outcome. For example, Nosarti et al (2008) using this method, found widespread grey and white matter abnormalities that were associated with a range of cognitive measures in preterm adolescents. Isaacs et al (2001, 2002, 2004), in neurologically normal preterm adolescents, were able to demonstrate an association between impaired calculation skills and subtle cortical abnormalities detected by VBM (Isaacs et al, 2001), an association between deficits in visuo-spatial skills and cortical abnormalities (Isaacs et al, 2002) and associations between subtle abnormalities in several brain areas and IQ scores (Isaacs et al, 2004).

3.3.3 Epilepsy

Although not very common in preterm children, the prevalence of epilepsy in preterm populations is higher than in the normal population (see below). The clinical details and, in particular, the possible underlying pathology associated with epilepsy in preterm children has been studied only infrequently in a systematic way. This may be because it is often assumed that there is no independent association between prematurity and epilepsy (Ellenberg and Nelson, 1979; Nelson and Ellenberg, 1986, 1987). The reported prevalence rates of epilepsy in very preterm born children vary from 0.6% (Aziz et al, 1995) to over 7% (Saigal et al, 2001), depending on details of the study design, such as the selection criteria of the subjects (e.g. gestational age groups, birth weight groups, presence of neuromotor impairment, type of brain lesion, presence of neonatal seizures, age at assessment) or whether it is a hospital or population based study.

Epilepsy has mainly been regarded as a concomitant disorder in children who develop major neuromotor impairments following preterm birth. It is clear that the prevalence of epilepsy is highest in children with disabling neuromotor impairments (i.e. severe spastic tetraplegia and hemiplegia) and that a seizure disorder frequently co-exists with other impairments such as impairment of cognitive function, hearing loss and loss of visual function (Nalin et al, 1990). For example, the HIFI Study Group (1990) reported a cumulative incidence of epilepsy of 3% up to the age of 2 years in infants with a birth weight between 750-2000 g, and the majority of the children had severe motor impairment. However, Dunn, Robertson and Crichton (1986), for example, reported a 4.2% cumulative incidence of epilepsy at the age of 6.5 years in a cohort of children with low birth weight (<2000 g) and the majority of these children did not have severe motor impairment. This suggests that even in preterms without major neurological impairments epilepsy is more frequent than in term born children.

Investigations of preterm populations without pre-selecting the infants who have major neuromotor impairment suggest that preterm children with epilepsy, when compared to preterm children without epilepsy, have a tendency for lower birth weight, lower gestational age (Nalin et al, 1990; Ishikawa et al, 1995; Amess et al, 1998), lower APGAR scores (Ishikawa et al, 1995; Nalin 1990), need for longer ventilation or oxygen dependency (Ishikawa et al, 1995; Amess et al, 1998) and had more often neonatal seizures (Otani et al, 1990). Nalin et al (1990) identified the occurrence of severe apneas (defined as repeated cessation of respiration, possibly cyanotic and requiring therapy) as a risk factor for epilepsy. However, the authors of this study did not discuss if the severe apneas might in fact have been neonatal seizures. In most of the above mentioned studies, the occurrence of epilepsy was associated with neurological and/or neurodevelopmental problems varying from very minor to severe. For example, in the study conducted by Amess et al (1998), Full Scale IQ (assessed with the Wechsler Scale) at the age of eight years was significantly lower in the children with epilepsy when compared to those who had no epilepsy.

Few studies (which consist mainly of neonatal cranial ultrasound) have investigated the associations between neuroimaging findings and epilepsy in preterm children. Comparisons between the few existing studies are problematic since definition and classification of ultrasound findings vary between these studies. A common finding, however, is that the majority of preterm infants who develop epilepsy have extensive white matter damage of mainly haemorrhagic origin, or often more than one type of lesion. Purely intraventricular haemorrhage seems not to carry a risk for epilepsy (Otani et al, 1990; Aziz et al, 1995; Amess et al, 1998). There are some reports that focus on infantile spasms in preterm infants, and these studies provide some evidence that there is an association between cystic periventricular leukomalacia identified by neonatal ultrasound and the occurrence of infantile spasms (Okumura et al, 1996, 2001).

However, to date there are no MRI studies later in infancy or childhood that investigate the structural correlates of epilepsy in preterm infants systematically without selecting subjects on the basis of neuromotor impairments (cerebral palsy) or severe brain lesions.

3.4 Imaging of the brain in the preterm newborn and child

Neonatal cranial ultrasound and more recently MRI in the neonatal period and later in infancy and childhood are the main imaging techniques used to identify brain lesions and to investigate relationships between brain structure and outcome in preterm infants.

Neonatal cranial ultrasound has been shown to be able to diagnose perinatally acquired brain lesions and, to a limited extent, predict very early in life the overall neurological and neurodevelopmental outcome. There are many studies that have shown an association between brain lesions detected by neonatal cranial ultrasound and long term outcome (see section 3.3.2). Neonatal ultrasound can reliably identify haemorrhagic and hypoxic-ischaemic lesions with parenchymal involvement (Hope et al, 1988; Fawer, Diebold and Calame 1987; de Vries et al, 1988). It is, however, limited in the detection of lesions such as subtle white matter abnormalities and early (non-cystic) PVL, as well as in detection of

the diffuse form of white matter damage (Maalouf et al, 2001), very small lesions or lesions that are located more peripherally (Hope et al, 1988; Adcock et al, 1998).

More recently, MR imaging of the brain has been used in the neonatal period. Visual analysis of conventional structural MRI, especially in combination with ultrasound (deVries et al, 1998), has been shown to improve further the identification of abnormalities (Maalouf et al 1999, 2001) and assessment of myelination, as well as improving prediction of neurodevelopmental outcome, in particular, for neuromotor function (Roelants-van Rijn, 2001). In addition, newly developed MR image acquisition techniques, such as diffusion weighted MR imaging (DWI) and diffusion tensor imaging (DTI), are now increasingly used to assess white matter structure in the neonatal period. For example, Counsell et al (2003) used diffusion weighted MRI to investigate further the nature of diffuse excessive high signal intensity in the white matter that had been previously identified on conventional T2 weighted imaging in preterm infants at term. The results of this study supported the hypothesis that these high signal intensities in the white matter are a correlate of white matter abnormalities rather than representation of a normal biological phenomenon. However, as in most of the MRI studies that have been performed in preterm infants at term age, long-term outcome data (beyond 2 years of age) are only emerging and therefore no definitive conclusions can be drawn with regard to the significance of these subtle neonatal MR findings and their relationship with function later on in life.

As already discussed in previous sections in this chapter, there are numerous studies that have been undertaken to investigate perinatally acquired lesions and the functional consequences with regard to neurological and neurodevelopmental status later in infancy, childhood and/or adolescence. Using conventional MRI combined with visual analysis of the images, it has been possible to establish relationships between major neurological impairments, e.g. cerebral palsy (e.g. Krägeloh-Mann et al, 1995), severe visual impairment (e.g. Cioni et al, 1997) and lesion location and extent on MRI. The predominant pattern of lesions in preterm children as identified by visual analysis of MR images consists of abnormalities mainly in the periventricular white matter, including abnormalities such as areas of high signal on T2 weighted images without reduction of white matter (which is

regarded as the correlate of gliosis and strongly associated with leg dominated bilateral cerebral palsy), periventricular white matter reduction/ ventricular dilatation either with or without high signal on T2 weighted images (associated with bilateral spastic cerebral palsy if bilateral, and with hemiplegia if unilateral, and depending on the degree of white matter reduction often combined with visual impairment and global developmental delay). Cortical grey matter lesions have been rarely described on conventional MRI in preterm children.

3.5 Detection of subtle brain abnormalities

As outlined above, some of the structural brain abnormalities that are associated with impairment of function are likely to be too subtle to be detected by purely visual analysis of conventional MRI. Voxel-based morphometry (VBM; see chapter 8 for details on the methodology) is a relatively new technique that has been developed to investigate structural differences of grey and white matter in the brain. It provides a powerful tool for analysing 3D structural MR data sets. VBM essentially compares segmented images of grey or white matter using statistical methods that are similar to those that have been used for analysis of functional MRI to identify and make inferences about regional differences. A great advantage of this method is that it allows user-independent objective assessment of the whole brain and is not limited to investigation of only pre-defined regions of interest.

VBM has been used to investigate brain structure in a variety of populations, including the investigation of changes in brain structure that occur in normal subjects during development (e.g. Sowell et al, 1999), but also in a number of patient groups. In many of these studies subtle structural differences between patients and controls were identified that had not been evident on visual analysis of the MR images. For example, Woerman et al (1999) investigated adolescents with juvenile myoclonic epilepsy who had normal MRI on visual inspection. VBM revealed abnormalities of cortical grey matter in 20% of the patients. Richardson et al (1997) examined a group of patients with partial seizures and focal cortical dysgenesis detected by visual analysis of MR images. Using VBM, they were

able to confirm the brain abnormalities and detect abnormalities beyond the areas with focal cortical dysgenesis. VBM has also been used in numerous studies to investigate structural brain abnormalities in developmental and/or psychiatric disorders. For example, Wright et al (1999) examined patients with schizophrenia and found regional grey matter differences between patients and controls. Abell et al (1999) used VBM to examine patients with autism and were able to demonstrate differences in grey matter density in various brain regions when compared to controls. Using VBM, Isaacs et al (2001) were able to identify subtle grey matter abnormalities in the left parietal lobe in neurologically normal preterm adolescents with impaired calculation skills, and abnormalities in the right extrastriatal cortex that were associated with deficits in visuo-spatial skills (Isaacs et al, 2003). In many instances, by using VBM for analysis of MR data, differences in brain structure have been revealed that would have gone undetected by purely visual analysis of the images.

3.6 Summary

As reviewed above, neuropathological studies and, more recently, neuroimaging studies suggest that, although the typical brain injury in the preterm infant is predominantly injury to the white matter, it is very likely that grey matter is affected as well. Such grey matter abnormalities may explain the occurrence of impairments such as epilepsy and cognitive impairment, both of which are regarded as grey matter disorders, in a spectrum of impairments in which there is predominantly white matter damage. The structural anomalies of grey matter may be too subtle to be detected on neonatal cranial ultrasound or by purely visual analysis of conventional MRI.

The work presented in this thesis seeks to investigate the underlying pathology of two types of impairments that occur, often in conjunction, in preterm children, i.e. epilepsy and impairment of overall cognitive function. The main focus of the work described in this thesis is on the investigation of the relationships between magnetic resonance imaging findings (from both visual analysis and analysis of MR data by VBM) and these two

clinical outcomes. Futhermore, perinatal data, data obtained from neurological, neuropsychological and neurophysiological assessments are examined. In addition, the application of VBM in a paediatric population and in patients with visible brain lesions is investigated.

Chapter 4: Study population and methodology

In this chapter, the study population and the methods that have been used in this study are described. In subsequent chapters, reference will be made to sections of this chapter.

4.1 Study population

The participants in this study are members of the follow-up cohort of preterm infants at University College Hospital (UCH), London. Since 1979, all infants born at fewer than 33 weeks of gestation who had been born at UCH or transferred to UCH within the first week of life, have been enrolled in a long-term prospective follow-up study with the aim of investigating relationships between neonatal cranial ultrasound findings and long term neurodevelopmental outcome. Each infant who is enrolled in the study receives serial cranial ultrasound examinations in the neonatal period (for a detailed description of the protocol see section 4.2.1). Neurodevelopmental assessments are carried out at term aequivalent age, at age 12 months corrected, at age 4 years, and at the age of 8 years. For the current study some of the data collected at the 8 year assessments were used. Age was corrected for gestation up to two years of age in the current study.At the follow-up assessments, the families are routinely asked about a history of seizures and the information is entered into a database. Infants of the cohort who were born between January 1983 and December1991 and who had developed epilepsy by the age of 8 years were included in the work described in this thesis. The prevalence of epilepsy of 4.3 % in the UCH cohort had been determined in an earlier postal questionnaire survey (Amess et al, 1998). In addition, a telephone enquiry was undertaken for the birth years 1983-1988 by Dr Phil Amess, and for the birth years 1989-1991 by the author. In both the postal questionnaire survey and the present study, epilepsy was defined as the separate occurrence of two or more apparently unprovoked seizures (Aicardi, 1990).

Children with epilepsy who had been identified by the procedure described above were then invited to take part in this present study. Twenty-eight children with epilepsy and their families initially agreed to take part in the study. Based on the prevalence of epilepsy of 4.3 % determined in the study by Amess et al (1998), for the birth years 1983-1991 (n=923 long term survivors, 81% of whom were seen at follow-up at the age of 8 years), it would be expected that 39 children had developed epilepsy by the age of 8 years. Therefore a number of children with epilepsy might have been missed in the current study and based on the findings of the study by Amess et al (1998) it is likely that a proportion of those have had brain lesions involving periventricular white matter.

Four of the 28 families who initially agreed to take part decided not to participate in the MRI and EEG assessments because they found it too difficult to come to the hospital for assessments, or because they felt that the children would find the assessments too distressing. These children were not included in this study (for clinical details of these children see appendix 1, tables A1 and A2).

In an earlier study (Amess et al, 1998) on this cohort that had the aim to investigate the associations between neonatal ultrasound findings and the occurrence of epilepsy, it had been shown that the majority of the children with epilepsy had lesions that involved the periventricular white matter. Since the primary hypothesis in the present study is to test whether, in preterm children with epilepsy and/or cognitive impairment who have white matter lesions identified by neonatal cranial ultrasound, additional, undetected grey matter damage is present, it was attempted to match controls as closely as possible for neonatal ultrasound findings. Out of the 33 control children without epilepsy who initially had agreed to take part in the study, 3 decided not to participate in MRI and EEG assessments. These children were not included in the study (for clinical details of these children see appendix 1, tables A1 and A2).

For quantitative MR data analysis (voxel-based morphometry, VBM) 16 MR data sets obtained from term born, neurologically normal controls from a database of MRI controls were used.

4.2 Assessments and collection of clinical data

The assessments in this study included a structured neurological examination, an interview, an MRI brain scan and interictal surface EEG recording. Appendix 1 (table A3) provides details on available and missing data. In children who did not want to come to two visits to the hospital, the interview and neurological examination were conducted at a home visit. All participants in the study were assessed clinically by the author, and data collection regarding family history, maternal medical history, medical and neurodevelopmental history of the child and various aspects of epilepsy was performed in a systematic way by the author (see appendix 2 for data collection sheets). Perinatal and neonatal clinical data, ultrasound data and some data obtained from neurological assessments at term age, cognitive assessments and tests of neuromotor function at the routine follow-up assessments at age eight years, were extracted from the database held at UCH.

4.2.1 Neonatal cranial ultrasound data

Each preterm baby enrolled in the follow-up study at UCH received serial cranial ultrasound examinations in the neonatal period. The cranial ultrasound protocol consists of daily scans on all infants during the first week of life, followed by weekly scans until discharge from hospital. The classification of ultrasound findings used at University College London Hospital is shown in appendix 3. Scans were performed using a mechanical sector scanning apparatus; from 1983 to 1986, a Diasonics DS1 scanner (Sonotron, GE Ultrasound Europe) and from 1987 onwards, an ATL Ultramark 4 (Advanced Technology Laboratory, Philips Medical Systems). In both cases 5 and 7.5 MHz probes were used and, in addition, the Ultramark 4 had a 10 MHz short focus probe for clarifying suspicious findings. The images were stored on videotape and were then reviewed by two experienced observers. Details of the neonatal cranial ultrasound findings were then coded and stored in the database.

For the purpose of the present study, ultrasound findings were categorised according to the presence or absence of periventricular tissue involvement. In haemorrhagic lesions, the maximum grade of haemorrhage determined the category when two different grades of haemorrhage were present. Post-haemorrhagic ventricular dilatation, atrophy and cysts secondary to haemorrhage were categorised with the primary haemorrhagic lesion. Since there is some evidence that moderate/severe ventricular dilatation that is still present at term without preceding haemorrhage is likely to represent periventricular white matter loss, this was classified as lesions with parenchymal involvement. In contrast, transient (i.e. not present on the last scan) mild ventricular dilatation without preceding haemorrhage was categorised as normal.

Definitions of cranial ultrasound categories for this study are as follows:

a) **Normal**
   (including transient minor/mild ventricular dilatation, not seen on the last scan)

b) **Haemorrhagic lesions without parenchymal involvement**
   Germinal matrix haemorrhage (GMH), intraventricular haemorrhage (IVH) including posthaemorrhagic ventricular dilatation/hydrocephalus

c) **Lesions with parenchymal involvement**
   Haemorrhagic parenchymal infarction (HPI), cystic periventricular leukomalacia (cPVL), any grade of haemorrhage if present in combination with cystic periventricular leukomalacia, moderate/severe ventricular dilatation still present on the last scan.

Table 4.1 below provides a summary of the neonatal ultrasound categories for both the children with epilepsy and the children without epilepsy. There was no significant difference between the group with epilepsy and the group without epilepsy in the distribution of the ultrasound categories (Fisher's Exact test, $p=0.8$). The individual ultrasound diagnoses of the study participants can be found in appendix 4. With regard to the group of children not included in the study, there were two children with non-parenchymal lesions (one with and one without epilepsy), and five children with

parenchymal lesions (three with and two without epilepsy). The ultrasound diagnoses of the excluded children are shown in appendix 1, table A2.

Table 4.1: Distribution of the neonatal ultrasound categories in the group with epilepsy and the group without epilepsy

Cranial ultrasound categories	**Epilepsy** **N=24** *(%)*	**No epilepsy** **N=30** *(%)*	Chi-square test, Fisher's Exact **p-value**[§]
Normal ultrasound n= 7	3 *(13%)*	4 *(13%)*	**0.8**
No parenchymal involvement n=21 (intraventricular haemorrhage)	8 *(33%)*	13 *(43%)*	
Parenchymal lesions (parenchymal haemorrhage, cystic PVL*) n=26	13 *(54%)*	13 *(43%)*	

*PVL= Periventricular leukomalacia
$ comparison between the group with and the group without epilepsy

4.2.2 Interview, classification of seizures, neurological examination and assessment of motor function

4.2.2.1 Interview

Clinical data with regard to family and maternal history, post-neonatal medical and neurodevelopmental history of the child, as well as data relating to onset of epilepsy, seizure semiology, seizure frequency and medication were collected in a systematic way via interview (for details, see data collection sheet in the appendix 2). If necessary, additional clinical information was obtained from the child's medical records.

4.2.2.2 Classification of seizures

Epileptic seizures were classified according to the 1981 classification published by the Commission on Classification and Terminology of the International League Against Epilepsy (Commission of the Terminology of the International League Against Epilepsy, 1981). In the current study, the classification is mainly based on the clinical seizure type (rather than on interictal EEG findings), and has been slightly modified (see data collection sheet, appendix 2). Based on the description of seizures that was obtained at the interview, the seizures were classified independently by the author and another paediatric neurologist (Prof BGR Neville). Neonatal seizures were defined as seizures occurring up to 44 weeks of gestational age (Mizrahi and Clancy, 2000). Infantile spasms were diagnosed on the basis of seizure semiology and, where this information was available, signs of hypsarrhythmia on EEG at the time of these seizures.

4.2.2.3 Neurological examination and assessment of motor function

All children underwent a standardised neurological assessment (Amiel-Tison and Grenier, 1986) at term age and the findings from these assessments were obtained from the database. This neurological assessment assesses neurosensory function and spontaneous motor activity, passive and active muscular tone, primitive reflexes such as palmar grasp reflexes, Moro- and ATN-reflex and adaptation to manipulation. For each item a score between 0 and 2 is assigned (0 = typical result, normal range; 1 = moderate abnormality; 2 = very abnormal). The aim is to form a synthesis of clusters of signs and symptoms, and there are three categories of outcome (for the UCH follow up study this has been slightly modified):

a) **Normal** = no abnormalities detected, findings in the normal range
b) **Equivocal** = mild/moderate abnormalities present
c) **Abnormal** = clear abnormalities in the areas assessed have been identified

At the age of eight years, neuromotor function had been routinely assessed in all children using the Test of Motor Impairment (TOMI; Henderson and Stott, 1987). The results of the TOMI assessments were extracted from the UCL follow-up database. The test requires the child to perform a series of motor tasks in a standard way. Age-appropriate tasks are grouped under three headings: Manual dexterity, ball skills, static and dynamic balance. Normative scores for each of the three subtests are converted from raw scores. The sum of all scores makes the total error score, ranging from the best to the worst performance between 0 and 16. At the follow-up assessment at 8 years of age a maximum error score of 16 was assigned to children whose motor impairment was too severe to perform the tasks.

In the context of this present study, a structured neurological examination was carried out in each participant by the author and findings were categorised as follows:

a) **Normal** = entirely normal neurological examination

b) **Suspicious** = unspecific signs such as asymmetry of muscular tone and/or reflexes, muscular hypotonia (no functional impairment), muscular hypertonia (without definite signs of spastic cerebral palsy as defined below)

c) **Abnormal** = clear signs of cerebral palsy (CP; defined as a disorder of movement and/or posture and of motor function due to a non-progressive lesion/abnormality in the immature/developing brain (Mutch et al, 1992; Krägeloh-Mann et al, 1993; SCPE, 2000)

CP subtypes were classified into the following categories (adapted and modified from SCPE, 2000):

- ***Bilateral cerebral palsy (BSCP)*:** both sides of the body affected

  Leg dominated (legs more affected than arms)

  Three limb dominated (one arm and both legs equally and more affected than the other arm)

  Four limb dominated (arms equally or more affected than legs)

- ***Unilateral cerebral palsy ("hemiplegia")*:** only one side of the body affected

The following clinical signs were recorded: *Spasticity* (increased muscular tone and abnormal reflexes, i.e. hyper-reflexia and pyramidal signs), *Ataxia* (movements are performed with abnormal force, rhythm, accuracy), *Dyskinesis* (recurring involuntary and uncontrolled movements; dystonic when dominated by hypokinesia and hypertonia; choreo-athetotic when dominated by hyperkinesia and hypotonia).

This CP classification was used for categorisation of neuromotor findings in this study, and also for analyses investigating associations between these CP types and aspects of epilepsy, cognitive function and imaging findings. It was not attempted to determine the level of functional impairment by using classification tools for gross motor function since this was felt to be beyond the scope of this thesis.

4.2.3 Psychometric assessments of overall cognitive function

As part of the routine follow-up assessments, cognitive function at age eight years was assessed with the Wechsler Intelligence Scale for Children-Revised (WISC-R, Wechsler, 1974). The results of these assessments are stored in the UCH follow-up database and were extracted to be analysed in the context of this study.

The WISC-R consists of several subtests, each measuring a different facet of intellectual ability. The subtests are split into a verbal scale (Verbal IQ, VIQ) and a performance scale (Performance IQ, PIQ). The verbal subtests are designed to assess a child's ability for verbal expression and grasp of verbal concepts and abstract reasoning. The sum of all the scores on this scale produce is the VIQ. The performance subtests consist of tasks that often require the child to "do" things in a given time limit. These subtests help to assess visual and spatial organisation and perceptual ability, and the sum of all the scores on this scale produces the PIQ. When the scores of all subtests are put together they provide an estimate of a child's general intellectual ability, in this case a Full Scale IQ (FSIQ). However, in atypical populations calculation of a Full Scale IQ is not meaningful since the FSIQ can be regarded as an approximate mean of VIQ and PIQ, and if there is a significant discrepancy

between VIQ and PIQ (as it can be expected in atypical populations) it is preferable to investigate VIQ and PIQ separately.

A score of 100 corresponds to the performance of the average child of a given age on that given scale. In this current study, the IQ scores were entered as continuous variables in the statistical analyses. In addition, for the purpose of qualitative description, the scores were divided according to the classifications by Wechsler into the following categories: "Very superior" >=130, "superior" 120-129, " high average" 110-119, "average" 90-109, "low average" 80-89, "borderline" 70-79, " below borderline" <=69.

4.3 EEG recordings

Interictal scalp EEGs were recorded on the same day as the MRI, either before or after MRI examination. Waking EEG was recorded over 30-45 minutes using the extended 10-20 ("10-10") system electrode placement. The nasion was used as the common reference electrode. Data acquisition and off-line analysis was done using Neuroscan 4.1. Filter settings were 100 Hz low pass and 0.5 Hz high pass. The EEGs were assessed independently by a neurophysiologist (Dr Stewart Boyd, who was blind to the clinical data) and by the author. The raw data were assessed for general activity, abnormal background activity (focal or diffuse slowing), isolated sharp waves or spikes, sharp wave or spike wave complexes.

In order to address the main questions (i.e. is there evidence of focal or widespread damage and/or is there evidence for epileptic activity) EEG findings were categorised as follows:

a) Normal
b) General abnormality (focal or diffuse slowing)
c) Isolated spikes/sharp waves, which could imply more focal tissue damage (Dummermuth, 1965).
d) Epileptic discharges (sharp wave/spike/sharp wave complexes)

4.4 Magnetic Resonance Imaging (MRI)

4.4.1 MR data acquisition

MR data were obtained using a 1.5 T Siemens Magnetom Vision system. For visual analysis, multislice datasets (coronal and axial T2 weighted images, TSE, TR 5700 ms, TE 90ms, 19 slices, slice thickness of 5 mm, matrix size 224x256, field of view (FOV) 200 mm) were acquired. With regard to analysing MR data quantitatively, 2-D datasets present the following problems: Two-dimensional multi slice imaging results in imperfections in the shape of the selected slice (slice edge artefacts) that cause signal variation in the third dimension. Second, voxel dimensions in slice direction are significantly greater than in-plane. Three-dimensional imaging avoids these problems by encoding the third dimension in the same way as in-plane pixel encoding. Slice edge effects can so be avoided and small overall voxel dimensions can be used. Therefore for quantitative analysis (VBM), T1 weighted three-dimensional datasets (3D Magnetization Prepared Rapid Gradient Echo; MPRAGE sequence), TR 10 ms, TE 4 ms, TI 200 ms, flip angle 12 ^{0}, 128 sagittal partitions in the third dimension with field of view (FOV) 160 mm, matrix size 256x256, voxel size 0.98x0.98x1.25 mm were obtained.

4.4.2 Visual assessment of magnetic resonance images

Visual assessment of the images was performed to identify any abnormalities that are visually detectable on MR images. Images were independently assessed by an experienced neuroradiologist (Dr K Chong, who was unaware of the clinical details of the children) and by the author. The images were reviewed according to a scoring system (developed by the author; for details see appendix 2) that paid particular attention to periventricular white matter and cortical and subcortical grey matter structures. Findings were categorised according to the presence or absence of white and/or grey matter abnormalities as follows:

a) **Normal MRI**

b) **Periventricular white matter abnormalities**

- radiological signs of gliosis only
(defined as abnormally high signal on T2 weighted images)
- mild/moderate white matter reduction (+/- gliosis)
- severe white matter reduction involving subcortical white matter WM
(+/- gliosis)

In addition, thinning of the corpus callosum was recorded.

c) **Grey matter abnormalities**

- cortical lesions
- basal ganglia and/or thalamus abnormalities
(either decreased size or abnormally high signal on T2 weighted images)
- hippocampal abnormalities
(either decreased size or abnormally high signal on T2 weighted images)

4.4.3 Quantitative assessment of magnetic resonance imaging data – Voxel-based morphometry (VBM)

Voxel-based morphometry (VBM) was used to quantitatively assess the MR images, with the aim of identifying structural abnormalities that may be too subtle to be detected by purely visual analysis of the images. VBM can characterise grey and white matter differences in structural MR scans. It allows for comprehensive identification of differences, not just in specific structures, but throughout the entire brain. It has been shown in a number of studies to identify subtle brain abnormalities that are not detectable on visual analysis. The VBM methodology, interpretation and classification of findings are described in detail in chapter 8.

4.4.3.1 Control subjects for VBM image analysis

3D datasets from 16 healthy neurologically and radiologically normal subjects (7 female, 9 male) from a database of controls were used as controls for the VBM analysis. The age range of the VBM controls was 84-180 months (median 145 months); the age range of the preterm subjects of whom datasets were analysed using VBM was 84-181 months (median 130 months).

4.5 Statistics

The following provides an overview of the statistical methods and the general approach that has been employed for this study. It has to be emphasised that the work presented in this thesis is mainly explorative in character and the main aim was to identify clinical observations and imaging measures that provide some prognostic information, without attempting to quantify risk or determine causation. For both of the outcomes (i.e. epilepsy and overall cognitive function) statistical analyses consisted of both univariate analyses and regression analyses. In some cases, for a closer inspection of associations between independent variables and the outcome variables, contingency tables were created for descriptive and detailed examination. The study population was relatively small in sample size. Statistical analyses on small sample sizes carry the risk of resulting in unreliable and unstable risk estimations. With regard to the regression analyses, the general approach was to decide, based on clinical knowledge, what important clinical questions to investigate and set up the regression model accordingly, adjusting for covariates as appropriate. Thus, as already mentioned above, rather than trying to quantify risk or infer causation, the logistic and linear regression analyses are focused on whether a single variable or a set of variables provide clinically useful prognostic information.

Statistical analyses were performed using SPSS for Windows, release 12.0.1 (SPSS INC, Chicago, Ill., USA) and SAS, release 8.1 and 9.1 (SAS Institute Inc., Cary, NC, USA).

In a first step, univariate analyses were performed to investigate associations between individual independent variables and the outcome variables. As indicated by the nature of the variables, the appropriate statistical tests were performed as follows: For investigation of univariate associations between the binary outcome variable "epilepsy" with categorical independent variables Chi-square test with continuity correction for small sample sizes, Fisher`s Exact test and linear-by-linear association for ordered categorical variables were used; Mann-Whitney U test and Kruskal-Wallis test were used for investigation of associations with ordinal independent variables. For investigation of univariate associations between the continuous outcome variables "IQ scores" with categorical independent variables, the Mann-Whitney U test and Kruskal-Wallis test were used, and for investigation of associations with continuous independent variables Spearman`s correlation coefficient was employed. For the exploratory analyses that investigated associations between epilepsy and cognitive function while taking into account neurological/neuromotor function and/or brain lesions identified on MR imaging, analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used.

In a second step, a series of regression analyses (logistic regression for the binary outcome variable "epilepsy", and linear regression within the General Linear Model (GLM) for the outcome variables "cognitive function", i.e. Performance IQ and Verbal IQ) were performed. Independent variables identified in univariate analyses as being associated with the respective outcome variables were considered for inclusion in the second step of analysis. To avoid overlooking important associations, the approach was liberal with regard to the inclusion of variables since variables may contribute to a regression in unexpected ways due to the likely complex inter-relationships among the variables. In addition, some variables, for which there is some suggestion in the existing literature and clinical evidence that they are important, were also included in the second step of the analysis. Before entering variables into regression analyses, correlation matrices were created to identify correlations between independent peri-and neonatal variables. The principal component scores were calculated for correlated independent perinatal and neonatal variables to obtain a reduced dataset of "derived" explanatory variables that are orthogonal (not correlated with each other) thus dealing with the problem of multi-collinearity. For some continuous

variables for which there was a suggestion that the relationship with the outcome variable may be non-linear (birth weight, gestational age, APGAR score at 5 minutes, duration of oxygen supplementation) transformation into categorical variables was performed so that assumptions for regression analyses were met. P-values, adjusted odds ratio (OR), and 95% confidence intervals (CI) for logistic regression, and t-values, p-values, regression coefficients (B) and standard errors (SE) of B for linear regression are reported.

Since the work presented in this thesis is of exploratory nature rather than strict hypothesis testing, the inductive approach by Fisher was adopted regarding the interpretation of p-values. A p-value of <0.01 was regarded as indicating strong evidence, a p-value between 0.01-0.05 as indicating evidence, and a p-value of 0.05-0.1 as indicating weak evidence.

4.5.1 Some considerations concerning the study design for investigation of associations between neuroimaging findings and epilepsy

For this study, all available patients of the preterm cohort of the birth years 1983-1991 who had developed epilepsy by the age of eight years were included, and then controls (no epilepsy) were selected to achieve balance with respect to white matter ultrasound abnormalities. This design was chosen since the primary hypothesis of this study is that in those children who have epilepsy and/or cognitive impairment, grey matter abnormalities, additional to the white matter lesions identified by neonatal cranial ultrasound, are present. However, there is a possibility that such a design may result in a reduction in the observed associations between some variables of interest and the outcome variable epilepsy since some factors of interest (e.g. MR imaging variables) may be associated with ultrasound findings. Thus, to investigate whether response patterns differed between ultrasound strata, some exploratory univariate analyses were performed to investigate the associations between epilepsy and patterns for three MRI categories ("grey matter abnormality", "white matter abnormality" and "periventricular white matter reduction") within ultrasound categories (which were treated as strata; 0=normal, 1=abnormal without parenchymal involvement, 2=abnormal with parenchymal involvement). These analyses were performed

in SAS release 8.1 and 9.1 (SAS Institute Inc., Cary, NC, USA) using the extended Mantel-Haenszel statistics. The extended Mantel-Haenszel Test is a test that is suitable for testing the null hypothesis of independence between two dichotomous variables using data from a population subdivided into L classes. It is a test that is traditionally used for a 2x2xL contingency table analysis and allows adjustment for the effects of stratification (Stokes, Davis and Koch, 1995).

The response patterns within ultrasound strata were similar for the MRI variables "grey matter abnormality" and "white matter abnormality", and "periventricular white matter reduction". There was no relevant difference between results when analysis was performed with adjustment and without adjustment for ultrasound category respectively (see table 4.2 below). Therefore it was concluded that it was justified to perform the subsequent more detailed statistical analyses investigating associations between MR findings and epilepsy without adjusting for ultrasound categories.

Table 4.2: Analyses examining associations between main categories for visual inspection of MR images and epilepsy performed adjusted for ultrasound categories and unadjusted for ultrasound categories

MRI categories	**Adjusted for ultrasound category** ***Mantel-Haenszel test p-value***	**Unadjusted for ultrasound category** ***Mantel-Haenszel test p-value***
Grey matter abnormality* epilepsy	0.1	0.08
White matter abnormality* epilepsy	0.031	0.037
Periventricular white matter reduction*epilepsy	0.009	0.003

Part II

Clinical characteristics of the study population and associations between clinical variables and outcome

Part II of this thesis consists of two chapters, and each chapter includes a discussion of the relevant findings. Chapter 5 includes first a section that describes the perinatal, neonatal, and neurological characteristics of the study population. Second, different aspects of epilepsy such as seizure type, age at onset of seizures, seizure frequency, medication and EEG findings are discussed. This is followed by a presentation of the results of statistical analyses investigating associations between clinical variables and the occurrence of epilepsy. Chapter 6 focuses on overall cognitive outcome of the study population as determined by psychometric assessment using the Wechsler Intelligence Scale for Children-Revised (WISC-R) and associations with epilepsy. In addition, the results of statistical analyses examining associations between clinical variables and overall cognitive outcome are presented.

Statistics

Statistical analyses in this part of the thesis include both univariate and regression analyses. The general approach to statistical analysis and the methods that were applied are detailed in chapter 4, section 4.5.

Chapter 5: Clinical characteristics of the study population and associations of clinical variables with epilepsy

In this chapter, first, clinical characteristics are described; these include perinatal and neonatal characteristics, neurological status, additional impairments, different aspects of epilepsy and the findings obtained from interictal surface EEG. Second, the results of univariate analyses investigating associations between clinical variables and manifestation of epilepsy are presented.

Details of each study participant's neurological findings, additional impairments, seizure type and findings obtained from interictal surface EEG are shown in appendix 5. For the analyses presented in this chapter, data of all 54 children (24 with epilepsy and 30 without epilepsy) that formed the final study group were included.

5.1 Perinatal and neonatal characteristics of the study population

Table 5.1 below provides an overview of the distribution of perinatal and neonatal variables for the whole study population and for the group with epilepsy and the group without epilepsy separately. Details on the distribution of the neonatal cranial ultrasound findings are given in chapter 4, section 4.2.1 and in appendix 4.

There was a difference in the proportion between the number of boys and girls in the group with epilepsy (boys n=16, girls n=8). In the group without epilepsy the numbers of boys and girls were equally distributed. There was no difference between the two groups for mode of delivery when this was categorised into "vaginal" and "caesarean section". However, there was a difference in the frequency between the two groups with regard to "emergency caesarean section" (see table 5.1). In the group without epilepsy only 9/30 (30%) of the children required oxygen supplementation beyond 37 weeks of gestation (GA), whereas in the group with epilepsy the percentage of children who needed oxygen

after 37 weeks GA (11/30, 46%) was not markedly different from the percentage of those who did not need oxygen supplementation after 37 weeks GA (13/30, 54%). The percentage of children with a history of hypoglycaemic events (blood glucose <1.6) was greater in the group with epilepsy than in the group without epilepsy (7/24, 29% versus 4/30, 13%).

Median gestational age, birth weight, APGAR at five minutes, frequency of babies having been small for gestational age, frequency of multiple births, time to onset of spontaneous respiration, frequency of babies with persistent ductus arteriosus, or insertion of ventriculo-peritoneal shunt in the neonatal period were similar in the two groups.

For statistical comparison of peri-and neonatal variables between the group with epilepsy and the group without epilepsy, see section 5.5 of this chapter.

Table 5.1: Perinatal and neonatal details of the whole study population and for the group with epilepsy and the group without epilepsy

	Whole study population (n=54) (% within whole study population)	**Epilepsy (n=24)** (% within epilepsy group)	**No epilepsy (n=30)** (% within non-epilepsy group)
Gestational age, weeks; median (min-max)	27 (23-32)	27 (23-32)	27.5 (24-32)
Birth weight, g; median (min-max)	1114 (560-1830)	1114 (560-1830)	1145 (613-1730)
SGA (n)	*3/54*	*2/24*	*1/30*
Gender, male:female, n	31:23	16:8	15:15
Multiple birth, n	21 (39%)	9 (38%)	12 (40%)
Twins	*19*	*9*	*10*
Triplets	*2*	-	*2*
Mode of delivery, n			
Vaginal	34 (62%)	15 (62%)	19 (63%)
Spontaneous	*31*	*14*	*17*
Forceps	*3*	*1*	*2*
Caesarean Section	20 (37%)	9 (38%)	11 (37%)
Emergency CS	*11(55%)*	*6 (67%)*	*5 (45%)*
(% within category caesarean section)			
APGAR at 5 minutes; median (min-max)	8 (0-9)	8 (0-9)	8 (0-9)
Time to onset of spontaneous respiration*, n			
< 2 min	27 (61%)	12 (63%)	15 (60%)
2-5 min	12 (27%)	4 (21%)	8 (32%)
6-30 min	4 (9%)	2 (11%)	2 (8%)
>30 min	1 (2%)	1 (5%)	0
Duration of O2 supplementation, n			
<= 37 w GA	34 (63%)	13 (54%)	21 (70%)
> 37 w GA	20 (37%)	11 (46%)	9 (30 %)
Hypoglycaemic episodes, n	11 (20%)	7 (29%)	4 (13%)
PDA, n	10 (19%)	4 (16%)	6 (20%)
Ventriculo-peritoneal shunt inserted in neonatal period, n	5 (9%)	2 (8%)	3 (10%)
Positive family history for epilepsy, n	10 (19%)	2 (8%)	8 (26%)

SGA=small for gestational age (<10th centile); PDA = persistent ductus arteriosus requiring drug or surgical treatment. *Information available for 44 children; information missing in 5 children with epilepsy and in 5 children without epilepsy

5.2 Neurological status, neuromotor function and additional impairments

5.2.1 Neurological status at term age, neurological status and neuromotor function at time of this study (age 9-13 years)

Table 5.2 shows the findings of the neurological assessment at term age and Table 5.3 the neurological status at the time of the current study and results of the Test of Motor Impairment (TOMI). Neurological findings of the individual subjects are detailed in appendix 5. Details of categories of neurological status at term age and at the time of this study as well as definition of the CP subtypes are given in chapter 4, section 4.2.2.3.

Table 5.2: Neurological status at term age

	Whole study population	**Children with epilepsy**	**Children without epilepsy**	**Chi-Square test§,****
	n=54	**n=24**	**n=30**	**p-value**
Neurological status at term age (% within "neurology at term")				0.63
Normal	14	6 (*43%*)	8 (*57%*)	
Equivocal*	9	6 (*67%*)	3 (*33%*)	
Abnormal*	31	12 (*39%*)	19 (*61%*)	
Equivocal/abnormal	40	18 (*45%*)	22 (*55%*)	0.89

* Definitions/details: see chapter 4, section 4.2.2.3.
** Comparison between the group with epilepsy and the group without epilepsy
§ Chi-square test, linear-by-linear association for the ordered variable "neurological status at term"

Table 5.3: Neurological status at time of the current study (age 9-13 years) and TOMI error scores

	Whole study population **n=54**	**Children with epilepsy** **n=24** *(%)*	**Children without epilepsy** **n=30** *(%)*	**Chi-square test, Mann-Whitney U test**, §** **p-value**
Neurological status at time of current study (% within "epilepsy group") Normal Suspicious* Abnormal (CP)*	 16 19 19	 4 (*17%*) 6 (*25%*) 14 (*58%*)	 12 (*40%*) 13 (*43%*) 5 (*17%*)	0.004
TOMI error score *** Median (min, max)	6 (0-16)	16 (0-16)	3 (0-16)	0.001

*** Information missing in 1 case; maximum score of 16 assigned to 7 children with epilepsy because motor impairment was too severe to complete TOMI (Test of Motor Impairment)

** Comparison between the group with epilepsy and the group without epilepsy

§ Chi-square test, linear-by-linear association for the ordered variable, "neurological status at time of current study"

Mann-Whitney U test

Neurological status at term age was not significantly different between the group of children who developed epilepsy and the group that did not develop epilepsy (Chi-square test, linear-by-linear association, p=0.63) Closer inspection of the distribution of the neurological status at term age between the two groups showed that there was no marked difference in the percentage of children who developed epilepsy and those who did not develop epilepsy in the category "normal ". However, in the categories "equivocal" and "abnormal" there was a marked difference. In the category "equivocal" a higher proportion of infants developed epilepsy (6/9, 67%). Interestingly, in the category "abnormal", a higher proportion (19/31, 61%) of infants did not develop epilepsy. When the categories "equivocal" and "abnormal" were pooled together, this difference was far less pronounced. This may reflect potential difficulties with the decision of assigning findings on this examination to the category "equivocal" or "abnormal".

Neurological status at the time of this study (age 9-13 years) was significantly different between the group with epilepsy and the group without epilepsy (Chi-square test, linear-by-linear association, p=0.004). In particular, in the group with epilepsy 14/24 (58%) of the children had clearly abnormal signs (cerebral palsy, CP) whereas in the group without epilepsy only 5/30 (17%) had CP.

Within the group with abnormal neurological status, the most frequent subtype of CP was leg dominated BSCP (8/19; 42%), followed by unilateral CP (hemiplegia, 5/19; 26%) and three limb dominated CP (4/19; 21%). Four limb dominated CP (arms equally or more affected than legs) was only seen in 2/19 children with CP (ML, PD) and both children had epilepsy. Within the subgroup of children with leg dominated BSCP, children who had epilepsy tended to have a more extensive form than the children without epilepsy, i.e. the upper limbs were more frequently/extensively affected in the group with epilepsy. Appendix 5 gives details on the CP subtype of each subject. Figure 5.1 shows the distribution of CP subtypes in the two groups.

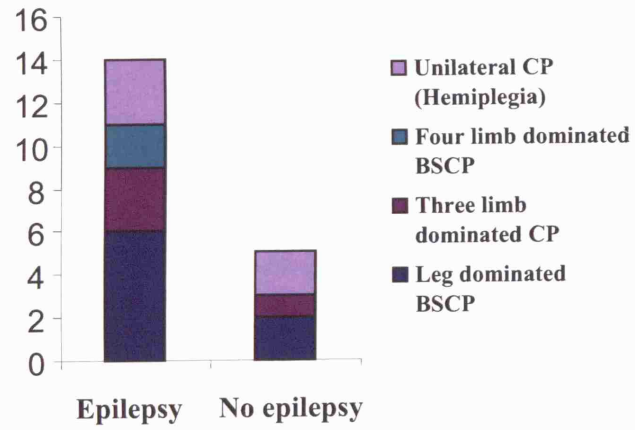


Figure 5.1: Distribution of CP subtypes (n=19 children with CP in the whole study population) between the group with epilepsy (n=14 children with CP; leg dominated BSCP n=6, three limb dominated CP n=3, four limb dominated BSCP n=2, unilateral CP n=3) and the group without epilepsy (n=5 children with CP; leg dominated BSCP n=2, three limb dominated CP n=1, unilateral CP n=2). The frequencies of cases in each subgroup are displayed on the Y-axis.

Table 5.4 below displays the neurological status in the two groups according to ultrasound categories. When examining the distribution of neurological findings within the three ultrasound categories, the three categories of neurological status were fairly equally distributed between the epilepsy and the non-epilepsy group in the category "normal" ultrasound. In the category "non-parenchymal lesion", the majority of the children with normal neurological status were in the group without epilepsy. In the ultrasound category "parenchymal involvement", the majority of the children with abnormal neurological status (CP) were in the epilepsy group, whereas the majority of the children with a "suspicious" neurological status were in the group without epilepsy.

Table 5.4: Neurological status at age 9-13 years according to ultrasound categories

	Neurological status at age 9-13 years	**Epilepsy** n=24	**No epilepsy** n=30
Normal ultrasound n=7	Normal*	2	2
	Suspicious*	1	2
	Abnormal (CP)*	0	0
Non-parenchymal lesion n=21	Normal	2	8
	Suspicious	3	4
	Abnormal (CP)	3	1
Parenchymal lesion n=26	Normal	0	2
	Suspicious	2	7
	Abnormal (CP)	11	4

*Definitions/details see chapter 4, section 4.2.2.3
CP=cerebral palsy

Neuromotor function was assessed with the Test of Motor Impairment, TOMI. For this study the summary error score was used. The median error score in the whole study population was 6 (min 0, max 16, see table 5.3 above). There was a significant difference (Mann-Whitney U test, p=0.001) between the two groups in the TOMI scores. This difference remained significant (Mann-Whitney U test, p=0.016) when the seven children who had been assigned the maximum error score because they were too severely impaired to perform the tasks were removed from the analysis. All these children were in the group

with epilepsy, and the median of the error score changed to 7 (min 0, max 16) after removing these seven children from the analysis.

Associations between overall cognitive function and neurological findings are presented in chapter 6.

5.2.2 Additional impairments – hearing and vision

Hearing function was impaired (impairment was defined as "requiring hearing aids") in four children (n=2 with epilepsy, n=2 without epilepsy). Ten children (n=4 with epilepsy, n=6 without epilepsy) had reduced visual acuity that had been corrected with glasses. Two children with epilepsy had severe visual impairment (registered as blind). Severe visual impairment was associated with abnormal neurological status (CP) in all children. Impaired hearing function was associated with CP in all but one child (LO, no epilepsy, suspicious neurological status). Appendix 5 shows the additional impairments for the individual subjects.

5.3 Clinical characteristics of epilepsy in the study population

In this section, different aspects of epilepsy and EEG findings in the study population are described.

5.3.1 Family history of epilepsy and history of maternal seizures

Epilepsy in first degree relatives was present in 8/30 children without epilepsy and in 2/24 children with epilepsy. In one child (TM) with epilepsy, there was a history of maternal epilepsy (generalised tonic-clonic seizures, also present in the grandmother).

5.3.2 Seizure type

Information on seizure type was collected by interview (see data collection sheet, appendix 2). In all cases the parents were asked to describe the seizures and the seizures were then categorised as described in chapter 4, section 4.2.2.2. Data obtained from interictal surface EEG recordings were used in an attempt to improve seizure classification with regards to identifying a possible focal onset of seizures.

Data on seizure semiology and information from EEG recordings were available for all 24 children with epilepsy. Appendix 5 shows the seizure types for the each individual child.

Focal seizures only were present in 2/24 children, focal onset seizures and generalised seizures were seen in 11/24. In four out of these 11 children, secondary generalisation after focal onset was assumed. Seizures for which, based on the available clinical data, there was no suggestion for a focal origin ("only generalised seizures") were seen in 11/24 children.

Figure 5.2 below shows the distribution of the different seizure types in the study population. It also shows the percentage of cases with a history of infantile spasms. The majority of children (18/24; 75%) had more than one seizure type. The predominant seizure types were complex-partial (12/24; 50%) and absence seizures (12/24; 50%). The category absence seizures includes typical (simple or complex) and atypical absences (simple or complex), which may contain a focal element. Typical absences fulfilling the clinical and EEG criteria according to the 1981 International Classification of Epileptic Seizures (i.e. seizure characterised by abrupt clinical onset and offset and an EEG discharge of symmetric 3 Hz synchronous spike-wave complexes) could be diagnosed in one child (TR, normal neurology, normal imaging findings on visual inspection of MRI and VBM), whereas in the other 11 children (n=1 normal neurology, n=3 suspicious findings, n=7 abnormal neurology; except one child, all had abnormal MRI on visual inspection, all had VBM-detected grey matter abnormalities) the most likely diagnosis was atypical absence seizures (i.e. more progressive clinical onset and offset, often associated with different and

variable EEG patterns; may contain a focal element). Other seizure types included generalised tonic-clonic seizures, which were seen in 6/24 (25%) children. Simple partial seizures were present in 3/24 (13%) children. Generalised clonic seizures, atonic, and generalised myoclonic seizures were seen in 2/24 (8%) respectively, and generalised clonic seizures in one child. A history of infantile spasms (diagnosis based on clinical signs and signs of hypsarrhythmia on EEG) was present in 4/24 (17%) children.

Out of the four children with normal neurology, one had focal seizures only and three had focal and generalised seizures. Of the six children with a "suspicious" neurological status, five had only generalised seizures, and one child had focal and generalised seizures. In the group with abnormal neurology (CP), focal or focal onset seizures were most common in children with leg dominated BSCP (5/6) and in children with hemiplegia (3/3). The two children with four limb dominated BSCP had generalised seizures without a clinically identifiable focal onset.

A history of neonatal seizures was present in 8/54 (15%) children and all eight children developed epilepsy. This subgroup is discussed in more detail in section 5.4 of this chapter.

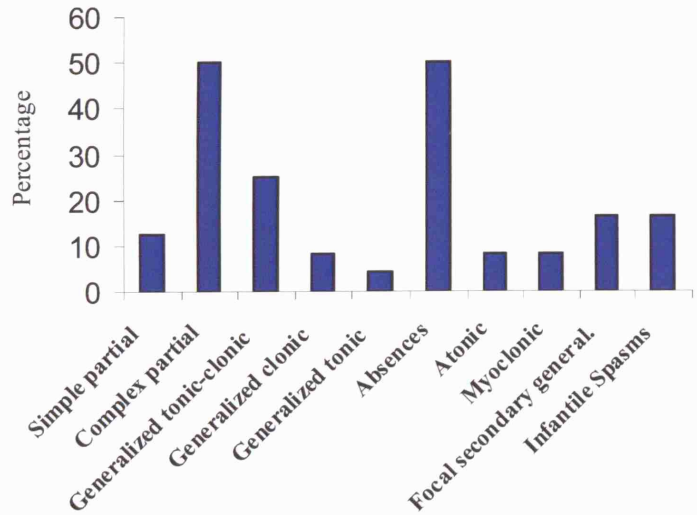


Figure 5.2: Distribution of seizure types in the epilepsy group (n=24) based on seizure semiology. More than one seizure type was present in 18 (75%) children. A history of infantile spasms was present in four children. The category absence seizures contains both typical (n=1) and atypical absence seizures (n=11).

5.3.3 Findings from interictal surface EEG recordings

Details on the classification of EEG findings for this study are given in chapter 4, section 4.3. EEG data were available for all 24 children with epilepsy and for 24 (80%) of the 30 children without epilepsy. Appendices 5 and 6 give details on the EEG findings for each individual child.

5.3.3.1 Findings on interictal surface EEG recordings in the whole study population

No abnormalities on EEG were found in 2/24 children with epilepsy (TM, SSk) and in 14/24 children without epilepsy.

In 18 (75%) of the 24 children with epilepsy focal/diffuse slowing or isolated sharp waves/spikes were seen. Focal/diffuse slowing or isolated sharp waves/spikes were present in 10/24 (42%) of the children without epilepsy.

Epileptic discharges, i.e. sharp wave/spike wave complexes, were see in one child in the group without epilepsy (in combination with background slowing, see below), whereas in the group with epilepsy epileptic discharges were seen in 9/24 (38%) children. In 4/9 children focal unilateral discharges (with generalisation in two cases) were seen, in 4/9 children bilateral discharges that were more pronounced on one side, and in one child generalised epileptic discharges (equally pronounced on both sides) were present. Both focal/diffuse slowing and epileptic discharges were seen in 5/24 (21%) children with epilepsy and in one child without epilepsy.

5.3.3.2 Associations of EEG findings with seizure type

In 4 of the 13 children with focal/focal onset seizures according to seizure semiology, focal epileptic discharges (sharp wave/spike wave complexes) were seen on the EEG recordings.

One child with focal seizures had bilateral generalised epileptic discharges. In 5/13 children only isolated sharp waves/spikes were seen, and in 2/13 children with focal onset seizures (TM, SSk) no abnormalities were identified on the interictal surface EEG. One child with focal onset seizures had only focal slowing of background activity.

In 4 of the 11 children with generalised seizures only (no focal onset according to seizure semiology), focal epileptic discharges were seen on EEG. In three of the four children the discharges were multifocal. No epileptic discharges but only isolated sharp waves/spikes were present in 5/11 children, and 2/11 had only some focal/diffuse slowing of background activity, and no epileptic discharges or isolated sharp waves/spikes.

5.3.3.3 Signs of laterality on EEG

Information on clinical focal signs during a seizure was only available for 3 of the 13 children with focal/focal onset seizures. In the remaining 10 cases the parents were able to give information on the presence of focal signs but could not remember the side on which focal signs were present during a seizure. In two (EG, SHay) of the three children for whom information on focal signs were available, the focal signs during a seizure corresponded with the focal signs of neuromotor findings. In only one case (EG) focal signs during a seizure, neurological findings, and the side of epileptic discharges were consistent.

In six of the ten children with focal onset seizures for whom no detailed information (body side of focal signs during a seizure) was available, EEG abnormalities were bilateral but more pronounced on one side.

In 7 of the 11 children with no identifiable focal onset on seizure semiology (i.e. the group with "only generalised seizures"), EEG abnormalities were more pronounced on one side. In two (AM, HJ) of these seven children neurological findings and side of EEG abnormalities corresponded to each other.

Appendix 6 provides details on signs of laterality in neurological findings, clinical focal signs of seizures, EEG abnormalities, visual inspection of MR images and VBM-detected grey matter abnormalities for each child.

5.3.4 Age at manifestation of epilepsy

Information on age at manifestation of epilepsy (after the neonatal period) was available for 23 of the 24 children with epilepsy. Age is given in months and corrected for gestation up to two years of age. Median age at epilepsy onset was 30 months (min 2, max 108 months). Seven of the 23 (30%) children had onset of epilepsy within the first 12 months of life, and by age 24 months epilepsy was manifest in almost half of the children (11/23; 48%).

For seven of the eight children with a history of neonatal seizures, information on age at manifestation of epilepsy was available. In all children with neonatal seizures, except in one child (BK) in whom onset of epilepsy was at 60 months of age, onset of post-neonatal seizures was within the first 24 months of life (median 9 months; min 2, max 60).

5.3.4.1 Association of age at manifestation of epilepsy with neurological status at term age and at age 9-13 years

Neurological status at term age showed a significant association with age at manifestation of epilepsy. Infants whose neurological findings had been classified "equivocal" (median 30 months; min 2, max 36) or "clearly abnormal" (median 12 months; min 5, max 108) had onset of epilepsy at a significantly earlier age than infants with normal neurological assessment (median 72 months; min 30, max 96) at term age (Kruskal-Wallis test, p=0.03).

Figure 5.3 below shows the associations between neurological status at the time of this study (age 9-13 years) and age at manifestation of epilepsy. There was weak evidence (Kruskal Wallis-test, p=0.07) for an association between neurological status at the time of

this study and age of epilepsy onset. As a group, children with abnormal neurological status (CP) had an earlier onset of epilepsy (median age 12 months, min 2, max 108) when compared with children whose neurological status had been classified as "normal" (median 54 months, min 30, max 72) and children who had a "suspicious" neurological status (median 36 months, min 18, max 96). Within the group of children who had clearly abnormal neurological findings (CP), age at onset of epilepsy varied according to CP subtype (see figure 5.3). For 13/14 children with CP, information on age at onset of epilepsy was available (information was missing for one (JW) child with leg dominated CP). All children with three limb (3/13) and four limb (2/13) dominated CP had manifestation of epilepsy within the first 12 months of life. In all five children with leg dominated CP onset of epilepsy was well after 24 months of life. In all three children with hemiplegia, epilepsy onset was within the first 24 months of life (in 2/3 within 12 months).

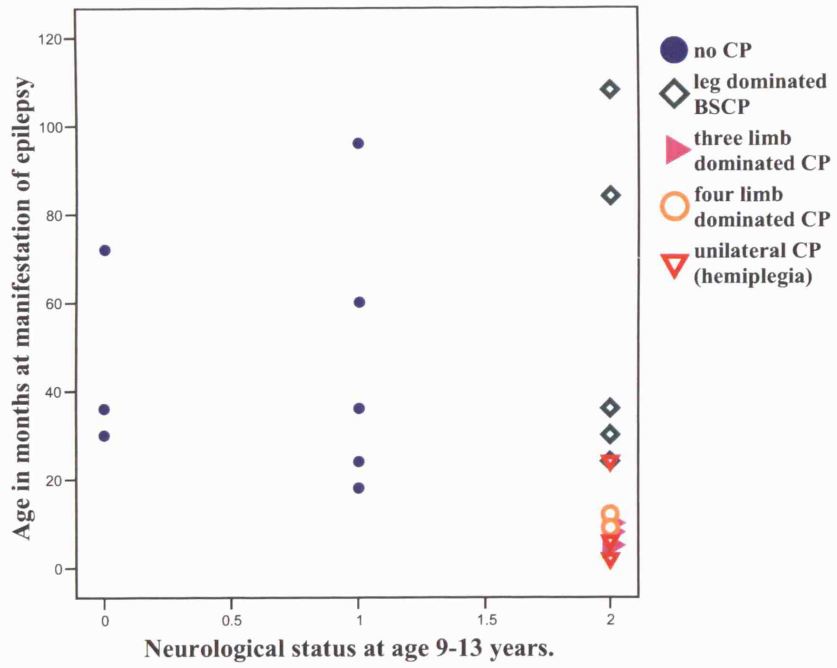


Figure 5.3: Association between age at onset of epilepsy and neurological status (variable coding: 0=normal, 1= "suspicious", 3=abnormal, cerebral palsy) at age 9-13 years. The group with CP is subdivided into leg dominated BSCP, three limb dominated CP, four limb dominated BSCP, and unilateral CP (hemiplegia). Age is corrected for gestation up to two years.

Three children (TO, TR, AP) with epilepsy had late onset of seizures, normal neurological status at term, normal neurological findings at the time of this study and Full Scale IQ in the normal range, normal neonatal ultrasound, normal MRI findings on visual assessment and no abnormalities on VBM analysis of grey matter (performed in two of the three children). The combination of those findings, and the fact that in these children the EEG findings were compatible with an idiopathic origin, suggest that these three children differ from the rest of the study population regarding the etiology of their epilepsy.

5.3.5 Seizure frequency and use of antiepileptic drugs

Information on the use of antiepileptic drugs was available for 23 of the 24 children with epilepsy. At the time of the current study, 10/23 (43%) children were treated with anticonvulsive drugs. Nine of these 10 children were treated with one drug only and one child (EF) with two anticonvulsive drugs. Two of the ten (20%) children who were on medication were seizure free (KS, TS). Both children had been seizure free for a short period of time, one child for four months and the other for six months. Eight of the 10 (80%) children on treatment were not seizure free. The seizure frequency ranged from 4-10 seizures a day to 1-3 seizures a year, with half the children having between 4-10 seizures a day to 1-3 per month.

Thirteen of the 23 children (57%) were not on medication at the time of the study. Ten of these 23 children had been treated at one time with anticonvulsive drugs and 3 had never been treated (2 of these children were seizure free at the time of the study for > 24 months, 1 child had 1-6 seizures per week). Eight of the 13 (62%) children without treatment were seizure free, with a median time of being free of seizures of 72 months (min 24, max 84). Five (38%) children without treatment were not seizure free and seizure frequency ranged from 1-3 seizures per day to 4-11 seizures per year, with over half of the children (3/5) having 1-6 seizures per year.

At the time of this study all four children with a history of infantile spasms were without anticonvulsive medication. All four children had previously had anticonvulsive treatment. All except one child (JW) in this subgroup were seizure free at the time of this study, and all three had not had any seizures for more than five years (ranging from 72-96 months).

There was no significant association between seizure frequency and ultrasound lesion (Kruskal-Wallis test, p=0.5), or seizure frequency and neurological status (Kruskal-Wallis test, p=0.9).

5.3.6 Discussion

The perinatal and neonatal characteristics of the study population are discussed in section 5.5.3 in the context of the results obtained from statistical analysis.

When neurological findings a term age were categorised in the three categories "normal", "equivocal", "abnormal" (according to a slightly modified classification of the Amiel-Tison assessment) more children who did not develop epilepsy were in the category "abnormal" neurology than in the group of children who did develop epilepsy, whereas it was the opposite in the category "equivocal". This may reflect problems of classification of findings that are not entirely normal into "equivocal" or clearly "abnormal" since there was no marked difference when the findings were only categorised in the two categories "normal" and "equivocal/abnormal". Looking at the whole group, including children with a history of neonatal seizures, neurological status at term in this study population was not associated with later epilepsy.

Neurological status at age 9-13 years and neuromotor function (as indicated by the TOMI scores) were significantly different between the two groups. Over half of the children with epilepsy had clearly abnormal neurological findings and were classified as having cerebral palsy. The finding that cerebral palsy in both preterm and term born children and epilepsy are associated is consistent with reports in the existing literature (see chapter 3, section 3.3).

When neurological status was compared within ultrasound categories, there was still a clear difference in the frequency of abnormal neurological status in the ultrasound category "parenchymal lesion" between the two groups. This supports the suggestion that in the group with epilepsy, the brain injury is likely to be more widespread, possibly involving both white and grey matter and probably too subtle to be detected by ultrasound. This is one of the main questions in this current study and it will be addressed in detail in the chapters dealing with the findings obtained from visual analysis of MR images and voxel-based morphometry analysis of grey matter segments.

The most frequent CP subtype (in the whole study population) was leg dominated BSCP, followed by hemiplegia (unilateral CP) and three limb dominated CP. This is not surprising given the pattern of white matter damage typically seen in preterm children (see chapter 3 for detailed discussion). In the group with leg dominated BSCP, the severity varied from minimal involvement of the upper limbs to marked involvement of the upper limbs and the more extensive forms were more frequent in the epilepsy group. Again, this suggests that the brain lesions are likely to be more widespread in the children who have epilepsy. It has to be kept in mind that the numbers in the CP subtype categories were small. However, the findings are in accordance with previous studies investigating epilepsy in patients with cerebral palsy, even if one takes into account that the definition of CP subtypes may vary between studies and the etiology of CP of patients included in the studies is often heterogeneous (Kwong, Wong and So, 1988; Hadjipanayis, Hadjichristodoulou and Youroukos, 1997; Carlsson, Hagberg and Olsson, 2003), in contrast to the current study, in which the brain pathology can be presumed to be fairly homogenous.

Age at manifestation of epilepsy was associated with neurological status at term age. There was also weak evidence for an association between neurological status at the age 9-13 years and age at manifestation of epilepsy, i.e. children with CP had an earlier onset than children with either normal or "suspicious" neurological findings. Within the CP group, the children with the most extensive forms of CP had early onset of epilepsy. The small numbers in the CP subcategories make it difficult to compare these findings with the existing literature. However, Carlsson, Hagberg and Olsson (2003), for example, in a sample of 164 children

with CP and epilepsy found a similar association between CP subtype and age at epilepsy onset. However, the finding that all children with hemiplegia in this current study had onset of epilepsy within the first 24 months of life is inconsistent with previous literature reporting that onset of seizures in children with hemiplegia often is late (e.g. Hadjipanayis, Hadjichristodoulou and Youroukos, 1997).

There is only very limited literature available on distribution of seizure types in preterm populations with epilepsy. In this current study, focal seizures or focal onset seizures were seen in about half of the children, and generalised seizures (no focal onset observed) also in about half of the epilepsy group. In a population with presumed focal brain lesions one would expect a higher proportion of focal seizures than observed in this study population. Since the classification of seizures into "focal" and "generalised" was based on seizure semiology, it is possible that a focal onset was missed in a proportion of children since focal onset prior to generalisation may not be apparent or witnessed. Interictal surface EEG showed focal discharges in four of the children that had been classified on the basis of seizure semiology as having generalised seizures without focal onset, and the discharges were multifocal in three of these four children. Previous studies have shown that in children with CP, various seizure types occur with a predominance of focal/focal onset with secondary generalisation seizures in hemiplegia (Aksu, 1990; Kwong, Wong and Son, 1998; Carlsson, Hagberg and Olsson, 2003) and generalised seizures in other types of CP (Hadjipanayis, Hadjichristodoulou and Youroukos, 1997).

Regarding more detailed classification of seizure types, it is surprising that in the current study, in which a fairly homogeneous type of underlying brain lesion can be assumed, many different seizure types were seen. However, this may be partly due to the fact that it can be difficult to differentiate between myoclonic, clonic, very brief tonic and atonic without ictal EEG or video EEG, and indeed, when interviewing the families it proved difficult in some cases to classify the clinical signs.

In agreement with previous studies, infantile spasms were frequent in this preterm study population. Okumura et al (1996), reported an association between severe forms of

periventricular leukomalacia diagnosed by MRI (PVL, in that study defined as periventricular abnormal high signal intensity on T2 weighted images and/or ventriculomegaly due to reduced periventricular white matter volume) and the occurrence of infantile spasms in a group of preterm infants with PVL. In the current study, all four children with a history of infantile spasms had CP and parenchymal involvement on neonatal ultrasound. The MRI findings of these children are presented in chapters 7 and 9.

In contrast to existing literature reporting associations between seizure frequency and ultrasound lesions and/or neurological status in children with symptomatic epilepsy, in this study population no clear associations between these variables were identified. However, in the current study a high proportion of the children who were on medication only had very infrequent clinical appointments for review of the clinical course and medication. Regardless of the neurological status, a high proportion of those without medication at the time of the current study had not been seen in clinic for a considerable time, and only a minority of the children with seizures had been seen by a paediatric neurologist. Therefore, it is difficult to draw firm conclusions from the available data with regards to associations between medication, severity of the epilepsy and association of seizure frequency with brain lesions and neurological status.

Interictal surface EEG recordings showed abnormal background activity in the sense of focal or diffuse slowing in the majority of the children with epilepsy and also in a high percentage of the children without epilepsy. Diffuse or focal slowing and isolated sharp waves/spikes have been shown to be associated with white matter damage (e.g. Dummermuth, 1965). Many of the children in this study have white matter damage, therefore the finding that a large proportion had these signs on EEG, irrespective of the presence or absence of epilepsy, is not surprising.

With regard to contribution to clarify a possible focal seizure origin in children without clear clinical focal signs and providing information regarding lateralisation of seizures, the EEG findings in this current study did not improve classification of seizures. However, it has to be kept in mind that a short interictal surface EEG recording, as was used in this

study, does have limitations regarding sensitivity and specificity and has low anatomical resolution.

5.4 Neonatal seizures

In this section, the subgroup of children with a history of neonatal seizures (defined here as clinical seizures up to 44 weeks of gestational age) is discussed in more detail and comparison is made with the subgroup of children who developed epilepsy but have no history of neonatal seizures. This section mainly aims to investigate whether, within the epilepsy group, the children with neonatal seizures differ from the group without a history of neonatal seizures in terms of perinatal/neonatal data and neurological outcome (data on cognitive outcome are presented in chapter 6). In addition, the aim is to investigate if the available data give any clue to a possible cause of the neonatal seizures, i.e. hypoxic-ischaemic events (global or localised), intracranial haemorrhage, structural brain abnormalities, intracranial infection, or metabolic disturbances. Furthermore, some aspects of epilepsy are compared between the two subgroups.

Information on the occurrence of neonatal seizures was obtained from the data base held at University College Hospital (UCH) and by interview with the families. Neonatal seizures had been recorded in eight children in this study population (see appendix 5). Information on time of onset of neonatal seizures was only available for two of the eight children (WS, onset at age 39 hours, and ML, onset at age 48 hours).

5.4.1 Perinatal and neonatal data, neurological status at term age and at age 9-13 years

5.4.1.1 Perinatal and neonatal characteristics and neonatal cranial ultrasound findings

Table 5.5 below shows perinatal and neonatal characteristics and neonatal cranial ultrasound findings in the subgroup with neonatal seizures and the subgroup without

neonatal seizures. There was weak evidence (Mann-Whitney U test, p=0.09) that median gestational age was different between the two groups, with median lower gestational age in the group with neonatal seizures. Distribution of birth weight, however, was similar in the two groups.

With regards to signs that may indicate problems in the perinatal period (in the sense of hypoxic-ischaemic events) only mode of delivery (i.e. information on necessity for emergency caesarean section) and APGAR score at 5 minutes were available for all subjects. Cord pH, and signs for fetal distress (e.g. fetal bradycardia or tachycardia, loss of variability on CTG recording) had been recorded insufficiently and therefore were not included in the analysis. APGAR score at 5 minutes was similar in the two groups. In all three children who had been delivered by caesarean section, an emergency caesarean was performed. However, the numbers were too small to draw any conclusions from comparison with the group without neonatal seizures. The time to onset of spontaneous respiration (information available for 6 children with neonatal seizures and for 13 children without neonatal seizures) may be an indirect signs of perinatal problems. There was no indication that this time was longer in the group with neonatal seizures when compared with the group without neonatal seizures. Although there was only weak evidence on statistical testing, the need for oxygen supplementation (Mann-Whitney U test, p=0.08) was slightly longer in the group with neonatal seizures. This may reflect a more severe neonatal course for the subjects in this group when compared with the groups without neonatal seizures. There was no case with intracranial infection (meningitis) in either group and there was no marked difference in the frequency of children with episodes of hypoglycaemia.

With regards to intracranial lesions detected on neonatal cranial ultrasound, there was a difference in the distribution of lesions between the two groups. Although there was only weak evidence for a difference on statistical testing (Fisher's Exact test; p=0.09), closer inspection of the distribution shows that all children in the neonatal seizure group had lesions on ultrasound, and the majority (7/8) had severe lesions involving the periventricular parenchyma. In contrast, in the group without neonatal seizures the

distribution between non-parenchymal and parenchymal lesions was more even (7/16 vs. 6/16).

Magnetic resonance imaging at the time of this study identified structural brain abnormalities (malformations) or brain lesions indicating focal ischaemia (different from the typical pattern seen in preterm infants) not diagnosed by neonatal ultrasound in three children in the neonatal seizure group, and in no child without neonatal seizures. Of these three children, two (ML, HJ) had a middle cerebral artery infarct and one (JW) a schizencephaly with polymicorgyria (see chapters 7, 8 and 9 for a more detailed discussion).

Table 5.5: Neonatal and perinatal characteristics and ultrasound findings; comparison between the group with neonatal seizures and the group without neonatal seizures

	Epilepsy – neonatal seizures **n=8**	**Epilepsy - no neonatal seizures** **n=16**	**Chi-Square test, Fisher's Exact test, Mann-Whitney U test** **p-value**
Gender, male:female, n	6:2	10:6	0.7
Gestational age, weeks; median (min-max)	26 (23-32)	28 (24-31)	0.09
Birth weight, g; median (min-max) *SGA (n)*	999 (634-1759) 0/8	1092 (860-1830) 2/16	0.5
Multiple birth, n *Twins*	5/8	6/16	1
Mode of delivery, n Vaginal *Spontaneous* *Forceps* Caesarean Section *Emergency CS* *(within category caesarean section)*	5/8 *5/5* - 3/8 *3/3*	10/16 *9/10* *1/10* 6/16 *3/6*	0.6 n/a
APGAR at 5 minutes; median (min-max)	7 (0-9)	8 (4-9)	0.6
Time to onset of spontaneous respiration*, n < 2 min 2-5 min 6-30 min >30 min	 5/6 0 0 1/6	 7/13 4/13 2/13 0	 n/a
Duration of O2 supplementation <= *37 w GA* > *37 w GA*	 2/8 6/8	 11/16 5/16	 0.08
Hypoglycaemic episodes, n	3/8	4/16	0.6
Neonatal cranial ultrasound category, n Normal Non-parenchymal Parenchymal	 0 1/8 7/8	 3/16 7/16 6/16	0.09

* Information available for 6 children with neonatal seizures and for 13 children without neonatal seizures

5.4.1.2 Neurological status at term age and neurological status at age 9-13 years

The findings from neurological examination at term age, at the time of the current study (age 9-13 years of age) and results of assessment of neuromotor function (TOMI) are shown in table 5.6 below. Information on neurological assessments at term age were available for all children and data on neuromotor function (TOMI) for all children with neonatal seizures and 15/16 children without neonatal seizures. For five of the eight children in the neonatal seizure group and for two children in the group without neonatal seizures, the maximum error score of 16 had been assigned for the TOMI since the severity of their motor impairment prevented them from completing the TOMI.

Neurological status at term age was significantly different (Chi-Square test, linear by linear association, p=0.01) between the two groups, with the majority (7/8) of the children who had neonatal seizures having clearly abnormal neurological signs at term age. No child in this subgroup had a normal assessment at term age. In contrast, in the group without neonatal seizures, distribution across the three outcome categories was almost even. Neurological status at the time of the current study (age 9-13 years) showed a similar distribution of outcome categories in the two groups as the findings at term age, with the majority of the children in the group with neonatal seizures having clearly abnormal neurology (cerebral palsy). Not surprisingly, the median of the TOMI error score was different between the two groups, although statistical testing showed only weak evidence (see table 5.6). For both assessments it has to be kept in mind that the numbers in the groups and cells were small, so any statistical result needs to be treated with caution.

When neurological status at the later time point (age 9-13 years) was compared within ultrasound categories, the outcome regarding neuromotor findings was similar for both groups. As described above, no child with neonatal seizures had a normal neonatal ultrasound and only one child had a non-parenchymal lesion. When the seven children with parenchymal lesions in the neonatal seizure group were compared with the six children with parenchymal lesions in the group without neonatal seizures, the majority in both groups (6/7 in the neonatal seizure group, 5/6 in the group without neonatal seizures) had

abnormal neurological findings (cerebral palsy) and only one child in each group was assigned a "suspicious" neurological status, i.e. unspecific neurological signs without functional impairment.

Table 5.6: Neurological status at term age, at time of the current study (age 9-13 years) and TOMI error scores. Comparison between the group with neonatal seizures and the group without neonatal seizures

	Epilepsy – neonatal seizures **n=8**	**Epilepsy - no neonatal seizures** **n=16**	**Chi-Square test#, Mann-Whitney U test** **p-value**
Neurological status at term*, n Normal Equivocal Abnormal	 0 1/8 7/8	 6/16 5/16 5/16	 0.01
Neurological status at time of this study (9-13 years)**, n Normal Suspect Abnormal	 0 1/8 7/8	 4/16 5/16 7/16	 0.04
$^**TOMI error score,** median (min-max)	16 (6-16)	8 (0-16)	0.09

* Details on categories see chapter 4, section 4.2.2.3
** Details on categories see chapter 4, section 4.2.2.3
# For the ordered variables "neurological status" linear-by-linear association was used
^ Information missing in one child in the group without neonatal seizures
$ No data available for 1 child without neonatal seizures; maximum score assigned because impairment was too severe to allow completing the test in 5/8 children with neonatal seizures, and 2/16 children without neonatal seizures

5.4.2 Clinical characteristics of epilepsy in the subgroup with neonatal seizures

All eight children with a history of neonatal seizures developed epilepsy. Information on age at manifestation of epilepsy (i.e. onset of post-neonatal seizures) was available for seven of the children and was significantly earlier in the group with neonatal seizures (median 9 months, min 2, max 60 months) when compared with the 16 children (median 36 months, min 8, max 108 months) who had epilepsy but no neonatal seizures (Mann-Whitney U test, p=0.007). This difference was far less pronounced when comparison was made only within the ultrasound category "parenchymal lesion" (Mann-Whitney U test, p=0.06). In all children with neonatal seizures, except in one child (BK) in whom onset of epilepsy was at 60 months of age, onset of post-neonatal seizures was within the first 24 months of life, and 5/7 children had onset of post-neonatal seizures within the first 12 months of life.

At the time of the current study 4/8 children still had seizures. The time period of being seizure free was over 60 months in 3 children, only 1 child (TS) had been seizure free for only a short time (6 months).

All children with a history of neonatal seizures had been treated with anticonvulsive medication at some point. However, except one child (EG), none of the children were treated with anticonvulsive medication at the time of the current study.

All children with a recorded history of infantile spasms had a history of neonatal seizures. One child (HJ) had only infantile spasms and did not develop any other types of seizures. Five of the eight children with neonatal seizures developed generalised seizures without an identifiable focal onset (based on clinical signs). Only in 2/8 children could a focal onset be identified, and one child had also generalised seizures. Three out of the eight children had more than one seizure type.

5.4.3 Discussion

This section summarises and discusses the main results obtained from a closer inspection of the subgroup with neonatal seizures and comparison of this group with the subgroup without neonatal seizures.

In the subgroup with neonatal seizures, the frequency of intracranial haemorrhage (haemorrhage with parenchymal involvement, in particular) diagnosed by neonatal cranial ultrasound was higher when compared with the group without neonatal seizures, and on MR imaging (see chapters 7 and 8) there was evidence for an extensive focal ischaemic injury (middle cerebral artery infarct) in two children and a brain malformation (schizencephaly) in one child. Gestational age was slightly lower in the neonatal seizure group. The available data did not suggest that perinatal hypoxic-ischemic events were more frequent in the neonatal seizure group. However, the need for of oxygen supplementation was longer in this group, suggesting a more severe and complicated neonatal course when compared to the group without neonatal seizures. The finding that gestational age was slightly lower in the neonatal seizure group is consistent with findings from previous hospital based studies (Scher et al, 1993; Ishikawa et al, 1995), which indicates that in very preterm infants the risk for neonatal seizures increased with lower gestational age. It has been shown that a major cause of neonatal seizures in preterm infants is intracranial haemorrhage, in particular, when the haemorrhage extends into the periventricular tissue (van Zeben-van der Aa et al, 1990; Strober, Bienkowski and Maytal, 1997). The findings in the current study are consistent with this.

Localised ischaemic events resulting from infarcts in major arterial vessels and brain malformations have been recognised as being strongly associated with the occurrence of neonatal seizures in both preterm and term born infants (Volpe, 2001). Indeed, MRI performed in the context of this current study revealed infarcts of the middle cerebral artery in two children and a brain malformation in one child with neonatal seizures.

Perinatal asphyxia and/or hypoxic-ischaemic events in the neonatal period have a strong association with neonatal seizures in both term and preterm infants (Holden, Mellits and Freeman, 1982; Ishikawa et al, 1995). The available data in the current study only allowed this to be investigated to a limited extent. For example, information on fetal distress (findings from CTG recordings, fetal heart rate), cord pH, impaired multiorgan function and data on possible adverse postnatal events were not sufficiently available for investigation in this study population. Using APGAR scores without other evidence of fetal distress or other signs of clinical depression in trying to establish a diagnosis of perinatal asphyxia/ hypoxic-ischaemic events has been shown to be problematic. However, using a combination of the need for emergency caesarean section, APGAR score at 5 minutes, and time of onset to spontaneous respiration may give an indication of the clinical status in the immediate postnatal period, and for these data there was no difference between the two groups. This suggests that hypoxic-ischaemic events in the perinatal period were not more frequent in the group with neonatal seizures and therefore are unlikely to be a cause of neonatal seizures in this study population. However, there was weak evidence that in infants in the neonatal seizure group the need for oxygen supplementation was longer, indicating a more severe neonatal course.

All children with neonatal seizures in this study population developed epilepsy. Subsequent isolated seizures are not common in infants who have neonatal seizures; in most cases later epilepsy is associated with neurological and cognitive impairment (Holden, Mellits and Freeman, 1982; Nelson and Ellenberg, 1987; van Zeben-van der Aa et al, 1990). The findings from the current study are in accordance with this, in that the children with a history of neonatal seizures had poorer neurological and cognitive (see chapter 6) outcome than the children without neonatal seizures. However, when the groups were compared within ultrasound categories, the neurological outcome was similar for both groups. This suggests that outcome with regards to neurological status is likely to be dependent on the underlying brain injury rather than the presence or absence of neonatal seizures and that the presence of neonatal seizures could probably be regarded as a marker for the extent of brain injury. MRI investigation of the brain gives a more detailed picture of the extent of brain

injury than neonatal ultrasound. For this study population findings from visual inspection of MR images and from VBM analysis are presented in chapters 7 and 8.

No infant with neonatal seizures had a normal neurological assessment at term age. Within the epilepsy group, neurological status at term age was significantly different between the group with neonatal seizures and the group without neonatal seizures, and this difference remained when comparison was made within the ultrasound category "parenchymal lesion" only. This suggests that in the neonatal period a useful predictor of outcome regarding subsequent epilepsy is a combination of neonatal ultrasound (in the absence of neonatal MRI imaging) and neurological status at term age.

Age at onset of post neonatal seizures was earlier in those with a history of neonatal seizures than in those without a history of neonatal seizures. However, this difference was less pronounced when comparison between the two groups was made within the ultrasound categories.

All children with infantile spasms had a history of neonatal seizures. In a study on epilepsies of neonatal onset, including 57 (term born) infants, Watanabe et al (1999) found that 41% of the 44 infants with symptomatic neonatal seizures developed infantile spasms and subsequently half of them developed focal epilepsy. They concluded that infants with symptomatic neonatal seizures may develop infantile spasms as a transient epilepsy syndrome in infancy.

Since there were few numbers in the subgroups, formal statistical testing was not possible. However, the available data did not suggest a different outcome between the group with and the group without neonatal seizures with regard to aspects of the later seizure type and seizure frequency

There are some limitations with regard to the available data in the current study. First, the diagnosis of neonatal seizures had been based on clinical observations and no continuous EEG recordings were available. The diagnosis of neonatal seizures, in the preterm infant in

particular, on purely clinical observation is problematic (see e.g. Volpe, 2001). Subtle seizures may be missed and, on the other hand, non-epileptic events may be diagnosed as seizures. Data on time of onset, duration of neonatal seizures and seizure semiology were not available (time of onset of seizures was recorded in the data base for only two patients). Furthermore, there were only limited data available that allowed detailed investigation of the occurrence of hypoxic-ischaemic or other adverse events that have been described in the existing literature as being associated with the occurrence of neonatal seizures. However, and although the numbers in the groups are small and thus the results of need to be treated with caution, the available data suggest that in this preterm population neonatal seizures are mainly associated with extensive haemorrhagic brain injury and secondly with focal ischaemic injury and brain malformation. In addition, the data suggest that the occurrence of neonatal seizures in a population of preterm infants may be an early clinical marker for poor outcome.

5.4.3.1 Implications for further statistical analyses

Neonatal seizures were observed only in the group of preterms who developed epilepsy. Therefore, this variable appears to be a "deterministic" variable in this study population and this has implications for the regression analyses performed in chapter 9. This is discussed in more detail in this chapter.

5.5 Associations between clinical variables and manifestation of epilepsy

In this section, the results of statistical analyses investigating associations between clinical variables and manifestation of epilepsy are presented. Details on the statistical approach taken in this study are given in chapter 4 (section 4.5.). Briefly, as a first step, univariate analyses were performed to investigate associations between individual clinical independent variables and the occurrence of epilepsy. The findings from these analyses are presented in this section. In a second step, which includes both clinical and MR imaging variables, regression analyses were performed to determine whether a single variable or a set of variables provide clinically useful prognostic information. The regression analyses are presented and discussed in chapter 9.

For the analyses presented here data of the 54 children (24 with epilepsy and 30 without epilepsy) who had complete assessments were included.

5.5.1 Selection of variables for investigation

Based on the small body of existing literature on epilepsy in preterm children (for review see chapter 3), on findings from epidemiological studies on risk factors for epilepsy in childhood (that in most cases include preterm and term born infants and rarely focus on preterm populations, e.g. Nelson and Ellenberg, 1986, 1987; Rocca et al, 1987a, 1987b, 1987c) and, in addition, on clinical observations in the population of the current study (see sections above), the following variables were chosen for investigation: Family history of epilepsy, maternal epilepsy/seizures, gender, gestational age, birth weight, being small for gestational age, mode of delivery, APGAR score at 5 minutes, time to onset of spontaneous respiration, hypoglycaemic events, history of intracranial infection (meningitis) in the neonatal period or later (before onset of epilepsy), neonatal seizures, persistent ductus arteriosus (PDA; requiring either surgical or drug treatment), duration of oxygen supplementation (as a surrogate measure of chronic lung disease if >37 w GA) and presence of a ventriculo-peritoneal shunt.

The following variables, some of which have been identified in existing epidemiological and/or hospital based studies as being associated with childhood epilepsy, were not included in the analyses since they had been insufficiently recorded for the study population: meconium stained fluid, abnormal cry in the delivery room, signs of fetal distress, cord pH and episodes of sepsis in the neonatal period. A history of febrile seizures has been identified in numerous previous studies (e.g. Annegers et al, 1997) as risk factor for childhood epilepsy. For the current study it has been attempted to collect information on the occurrence of febrile seizures by interview. None of the children without epilepsy had a history of febrile seizures. A history of seizures associated with raised temperature was reported by the parents for seven of the children with epilepsy. However, it proved difficult to decide on the basis of the information gathered via interview with the parents/carers whether the described episodes were "febrile seizures" (i.e. occasional seizures occurring in association with fever but without evidence of intracranial infection or other definable causes), whether these episodes occurred before the first afebrile seizure, or whether the episodes were in fact first seizures in the onset of epilepsy during which the child was unwell and may possibly have had raised temperature. Therefore it was decided not to include this variable in the analysis. Neonatal cranial ultrasound findings have not been included in the univariate analysis since it had been attempted to match the two groups as closely as possible for this variable (for details see chapter 4).

5.5.2 Results

Table 5.1 (section 5.1) displays frequency distribution of the categorical variables and median of the continuous variables under investigation for the whole study population and for the two groups, the children with epilepsy and children without epilepsy, separately.

The only variable that showed a strong association with epilepsy was a history of neonatal seizures (Fisher's Exact test, p=0.001). The following variables were not significantly different in their distribution between the group with epilepsy and the group without epilepsy: gender (Chi-square test, p=0.3), birth weight (Mann-Whitney U test, p=0.5),

gestational age (Mann-Whitney U test, p=0.5), multiple birth (Fisher's Exact test, p=0.6), APGAR at 5 minutes (Chi-square test, p=0.3), mode of delivery (Fisher's Exact test, p=0.5), time to onset of spontaneous respiration (Mann-Whitney U test, p=0.9), oxygen supplementation >37 w GA (Chi-Square test, p=0.2), presence of arterial duct requiring drug or surgical treatment (Fisher's Exact test, p=1), and episodes of hypoglycaemia (Fisher's Exact test, p=0.5).

Since the numbers were too small for meeting the requirements of the statistical tests, no testing was done on the following variables: A ventriculo-peritoneal shunt was seen in 2/24 children in the epilepsy group and 3/30 children without epilepsy. A positive family history for epilepsy was more frequent in the group without epilepsy (8/30) than the group with epilepsy (2/24). There was one case of maternal epilepsy in the epilepsy group, and no case with a history of intracranial infection (meningitis) in the study population.

5.5.3 Discussion

In the univariate analysis performed on the whole study population, the only variable that showed a strong association with epilepsy was a history of neonatal seizures. Most studies investigating risk factors for childhood epilepsy do not specifically focus on preterm infants. Nevertheless, this finding is consistent with existing epidemiological studies on risk factors for childhood epilepsy (e.g. Nelson and Ellenberg 1986, 1987), which identified neonatal seizures, in particular when associated with structural brain abnormalities (Nelson and Ellenberg, 1986), as one of the main antecedents of childhood epilepsy, along with a positive family history or maternal history for epilepsy, brain malformations, neonatal meningitis and febrile seizures. In preterm infants, the occurrence of neonatal seizures associated with severe degrees of intracranial haemorrhage has been shown to be associated with later epilepsy (Watkins et al, 1988; van Zeben–van der Aa et al, 1990; Otani et al, 1990) and the findings in the current study are consistent with this

There was no strong association between the other perinatal variables examined and epilepsy, which is in agreement with the results of the population based studies conducted by Nelson and Ellenberg (1986, 1987), and, in addition, hospital based studies that focus on preterm infants (e.g. Watkins et al,1988). However, Ishikawa et al (1995) investigated a cohort of infants with a birth weight <1500 g and found an association between epilepsy and decreasing gestational age and decreasing birth weight, low APGAR score, duration of oxygen supplementation and nutritional problems. Ishikawa et al (1995), however, did not examine neonatal brain lesions, which may be associated with some of these variables, and the number of patients was very small.

Nalin et al (1989), in an epidemiological study including a large group of preterm infants, also identified severe prematurity as being strongly associated with later epilepsy, and Saliba et al (2001) identified in a large epidemiological study an association between decreasing birth weight and epilepsy in preterm infants. One possible explanation for the discrepancy between the findings in the current study and some of the studies mentioned above could be that in the current study only preterm infants born <33 weeks of gestation were investigated, whereas in the studies conducted by Nalin et al (1989) and Saliba et al (2001) preterm infants born up to 36 weeks of gestation were included, and in the study by Ishikawa et al (1995), the selection criterion was birth weight rather than gestational age and therefore included infants born >32 weeks of gestation.

5.6 Conclusions

In this study population, epilepsy was significantly associated with abnormal neurological status (cerebral palsy, CP) as assessed at age 9-13 years and with impairment of motor function as assessed with the TOMI. In contrast, the neurological assessment at term age showed no associations with later epilepsy, possibly indicating low sensitivity of the assessment, or alternatively, that at this early time abnormal neurological signs had not yet emerged. However, a significant association was seen between abnormal neurological status at term age and early onset of epilepsy. Children with abnormal neurological status at

age 9-13 years, tended to have earlier onset of epilepsy than those with normal neurological status or those who had been assigned a suspicious neurological status. Within the group of children with abnormal neurology, those with more extensive forms of bilateral CP (i.e. when all four limbs were involved) and those with hemiplegia had early onset of epilepsy, whereas in those with bilateral leg dominated CP ("diplegia"), age at onset was comparatively late, which might be a reflection of the extent of brain injury.

Focal onset seizures and seizures for which no focal onset could be identified were distributed similarly across the groups, which is unexpected in a population in which focal brain injury is assumed. Infantile spasms were frequent and all children with infantile spasms had parenchymal lesions on neonatal ultrasound. EEG identified in a large proportion of the study population (irrespective of epilepsy) abnormal background activity, which is likely to be seen as a reflection of the high frequency of white matter lesions in the study population. All children with neonatal seizures developed epilepsy and had large lesions on neonatal ultrasound. They had slightly lower gestational age and there was a tendency for longer need of supplementary oxygen, indicating that this subgroup might have been more vulnerable and had a more severe neonatal course compared to those without neonatal seizures.

Univariate analyses showed a strong association only for neonatal seizures and epilepsy; no other of the examined perinatal or neonatal variables were significantly associated with the manifestation of epilepsy, which might partly be explained by the fact that the group without epilepsy was selected to achieve balance between the groups with regards to ultrasound diagnosis. Furthermore, the numbers in this current study are rather small so that some of the statistical results have to be treated with caution and remain tentative.

Chapter 6: Cognitive function and associations between clinical variables and cognitive outcome

In this chapter, the study population's profile of overall cognitive function as indicated by IQ scores on the Wechsler Scale-Revised (WISC-R) is described. For the group of children with epilepsy associations between overall cognitive outcome and some aspects of epilepsy such as age at onset of epilepsy, seizure frequency and seizure type are discussed. Results of statistical analyses investigating associations between clinical variables and cognitive outcome in the whole group are presented.

Associations between overall cognitive function and findings obtained from MRI investigations and results obtained from regression analyses including both clinical and imaging variables are presented in chapter 10.

Details of each study participant's IQ scores obtained from psychometric testing at the age of eight years using the WISC-R are shown in appendix 5. For the analyses presented in this chapter, data from 44 children are included (15/24 children with and 29/30 children without epilepsy).

6.1. Perinatal, neonatal and neurological characteristics of the children included in the analyses; comparison with the excluded children

In this section, an overview is given of clinical characteristics of the group of 44 children included in the analysis of cognitive function. Table 6.1 below shows the clinical characteristics of these children.

Table 6.1: Clinical characteristics and neonatal ultrasound findings of the 44 children included in the analyses of cognitive function as indicated by IQ scores on the WISC-R

	% within study population (n=44)
Gestational age, weeks; median (min-max)	28 (23-32)
Birth weight, g; median (min-max)	1128 (560-1830)
SGA (n)	*2*
Gender, male:female	24:20
Multiple birth, n	17 (39%)
Twins	*15*
Triplets	*2*
Mode of delivery , n	
Vaginal	31 (70%)
Spontaneous	*29*
Forceps	*2*
Caesarean Section	13 (30%)
Emergency CS	*6 (46%)*
(% within category caesarean section)	
APGAR at 5 minutes; median (min-max)	8 (0-9)
Time to onset of spontaneous respiration*	
< 2 min	21 (58%)
2 - 5 min	12 (33%)
6-30 min	3 (8%)
Duration of O2 supplementation, n	
<= 37 w GA	29 (66%)
> 37 w GA	15 (43%)
Neonatal seizures, n	3 (7%)
Hypoglycaemic episodes, n	3 (7%)
PDA , n	9 (20%)
Ventriculo-peritoneal shunt inserted in neonatal period	3 (7%)
Ultrasound diagnosis (category), n	
Normal	6 (14%)
Non-parenchymal lesion	20 (45%)
Parenchymal lesion	18 (41%)
Neurological status at term age, n	
Normal	13 (30%)
Equivocal**	7 (16%)
Abnormal**	24 (55%)
Neurological status at time of the current study, n	
Normal	15 (34%)
Suspicious**	19 (43%)
Abnormal (CP)**	10 (22%)
TOMI error score[$], median (min-max)	4 (0-16)
Severe visual impairment**	-
Severe hearing impairment,** n	2 (5%)
Epilepsy, n	15 (34%)

SGA=small for gestational age (<10th centile); PDA = persistent ductus arteriosus requiring drug or surgical treatment. *Information available for 36 children. ** Definitions/details see chapter 4, section 4.2.2.3.
[$] Maximum error score of 16 assigned in one child with epilepsy (WS) because motor impairment was too severe to complete the TOMI

Ten children (19%) of the 54 from the original group had to be excluded from the analyses that investigated cognitive function. Nine of the 10 children who were excluded from the analyses had epilepsy (i.e. 37% of those with epilepsy were excluded). Thus, 15 children (63%) with epilepsy were included in the analysis. Two children were excluded because they did not have an assessment of cognitive function at age 8 years (JC, no epilepsy; TM, epilepsy). Eight (15%; ML, AU, SHay, TS, HJ, JW, EG, EF; all in the group with epilepsy) children were excluded from the analyses because they had not been able to complete the required number of verbal and/or performance tests needed for computation of an IQ score due to the severity of their neurolomotor impairments.

There was no significant difference between those included and those excluded with regards to gestational age (Mann-Whitney U test, p=0.5), birth weight (Mann-Whitney U test, p=0.8), multiple birth (Fisher's Exact test, p=0.9), APGAR score at 5 minutes (Mann-Whitney U test, p=0.7), duration of oxygen supplementation (Fisher's Exact test, p=0.5), proportion of babies with persistent ductus arteriosus (Fisher's Exact test, p=0.6), hypoglycaemic events (Fisher's Exact test, p=0.4), or neurological status at term (Fisher's Exact test, p=0.3). In the group of children excluded from the analyses one child, and in the group of children included two children, had been small for gestational age (SGA) at birth. The proportions of emergency caesarean sections and neonatal seizures were significantly different between the excluded group (neonatal seizures 50%, emergency caesarean section 50%) and the group of children included (neonatal seizures 7%, emergency caesarean section 14%. Fisher's Exact test, neonatal seizures p=0.003; emergency caesarean section p=0.021). It has to be kept in mind though that the statistical analyses regarding the above variables were performed on very small numbers.

Seven of the 10 excluded children were boys and 3 were girls. Gender was approximately equally distributed in the group of children whose data were included in the analysis (boys n=24, girls n=20).

There was a difference in the distribution of ultrasound lesions between the excluded and the included children (Fisher's Exact test, p=0.05). The majority (8/10) of the excluded

children had parenchymal lesions on neonatal cranial ultrasound. In contrast, in the group of children included in the analysis, parenchymal (n=18, 41%) and non-parenchymal (n=20, 45%) lesions were approximately equally distributed. Six of the 44 (14%) children had normal ultrasound findings. Within the group included in the analysis, the proportions of the three ultrasound categories were similar in the subgroup with epilepsy and without epilepsy (epilepsy: normal ultrasound 13%, non-parenchymal lesion 47%, parenchymal lesion 40%; no epilepsy: normal 14%, non-parenchymal lesion 45%, parenchymal lesion 41%).

In the group of excluded children all but one child had abnormal neurological findings (CP). Neurological status at the time of the current study was significantly different between the group of excluded children when compared to the group of children who were included in the analysis (Fisher's Exact test, p=0.001). Similarly, neuromotor function as indicated by the TOMI error scores was significantly poorer (Mann-Whitney U test, $p<0.001$) in the group that was excluded. Two of the 10 excluded children had severe visual impairment (i.e. child is registered as blind) whereas no child included in the analysis had such a severe visual impairment. Hearing impairment was present in 2/44 children who were included in the analysis and in 2/10 children who were excluded.

Nine of the excluded children had epilepsy. There was no significant difference between those 9 children and the 15 children with epilepsy included in the analysis with regards to seizure frequency (Mann-Whitney U test, p=0.8) or age at onset of epilepsy (Mann-Whitney U test, p=0.5). In addition, the frequency of multiple seizure types was not significantly different (Mann-Whitney U test, p=0.3) neither was there a significant difference (Chi Square test, p=0.4) in the distribution of focal (with or without generalisation) and generalised seizures (i.e. seizures in which a focal onset could not be identified by semiology and interictal surface EEG).

6.2 Overall cognitive outcome and IQ profile of the whole group

The purpose of this section is to describe the overall cognitive outcome (as indicated by performance on testing with the WISC-R) and the IQ profile of the 44 children for whom data from psychometric assessments were available. In addition, differences between PIQ and VIQ are discussed with regard to neurological status, performance on the TOMI and ultrasound categories.

In a normal population, the Full Scale IQ (FSIQ) can be regarded as an approximate mean of VIQ and PIQ. As outlined in chapter 4, the calculation of an FSIQ is not very meaningful in atypical populations because in such populations differences between VIQ and PIQ are often present. If there is a large discrepancy between PIQ and VIQ (according to Wechsler on average a difference of 12 IQ points between VIQ and PIQ is required for significance at the 5 percent level and a difference of >= 15 points is important and should be investigated further), as may be expected in atypical populations, it is preferable to investigate PIQ and VIQ separately. Thus, FSIQ is described and shown in the figures but not included in statistical analyses, which consider PIQ and VIQ separately.

The IQ scores were entered into the statistical analyses as continuous data. In addition, for the purpose of qualitative description, the scores were divided according to the classifications by Wechsler, 1991, into the following categories: "Very superior" >=130, "superior" 120-129, " high average" 110-119, "average" 90-109, "low average" 80-89, "borderline" 70-79, " below borderline" <=69.

6.2.1 IQ scores in the whole group

Figure 6.1 shows the median, minimum and maximum for FSIQ, PIQ and VIQ on the WISC-R for all 44 children. The median and non-parametric tests were used in these analyses since the psychometric data in the sample of the current study are not normally distributed and the sample sizes are relatively small. For the purpose of qualitative

comparison with a normative sample, the mean/median for a typical population is displayed in the figures. In a normative sample, in which the data are normally distributed, the mean and the median are the same. Therefore it can be assumed that the mean of 100 in a normative sample corresponds to a median of 100.

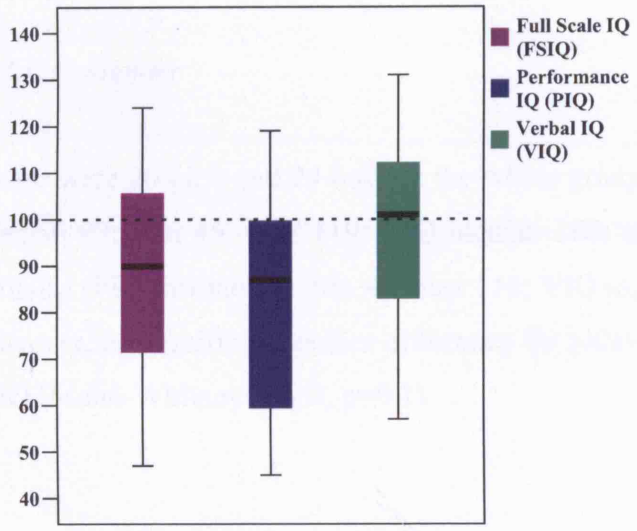


Figure 6.1: Full Scale IQ (FSIQ), Performance IQ (PIQ) and Verbal IQ (VIQ) on the WISC-R for the whole study population. Displayed is the median (solid black horizontal lines), minimum and maximum (whiskers) and interquartile range (25%-75%) represented by the boxes. IQ scores are displayed on the Y-axis. The dotted black horizontal line displays the mean/median of 100 for a normative sample.

For all three scales, there was a large variation in IQ scores (see figure 6.1). The median FSIQ in the whole group was 90 (min 47, max 124), which is at the lower limit of the average range. Twenty eight children (64%) had an FSIQ below the mean/median of 100 for a normative sample. Two (5%) of the 44 children had an FSIQ above 119, 28 (64%) an FSIQ between 80-119; in 4 children (9%) FSIQ was between 70-79, and 10 (23%) had an FSIQ below 70.

The median PIQ in the whole group was 87 (min 45, max 119), which is at the upper end of the low average range. Thirty three children (75%) had a PIQ below the mean/median of 100 for a normative sample. One of the 44 children had a PIQ of 119; 25 children (57%) had a PIQ between 80-119; 6 (14%) between 70-79, and in 12 (27%) children the PIQ was below 70.

The median VIQ in the whole group was 101 (min 57, max 131), which is well within the average range. Twenty-one children (48%) had a VIQ below the mean/median of 100 for a normative sample. Three (7%) of the children had an IQ above 119, 31 (70%) children had a VIQ between 80-119; 3 (7%) had a VIQ between 70-79, and in 7 (16%) children the VIQ was below 70.

6.2.1.1 Gender

There were 20 girls and 24 boys in the whole group. The median IQ scores for males (PIQ median 93; min 45, max 119; VIQ median 106; min 60, max 130) were higher than for females (PIQ median 84; min 46, max 118; VIQ median 95; min 57, max 113). There was, however, no significant gender difference for either PIQ (Mann-Whitney U test, p=0.6) or VIQ (Mann-Whitney U test, p=0.1).

6.2.1.2 Differences between Performance IQ and Verbal IQ

In 25 of the 44 children (57%), the difference between median PIQ and median VIQ was >=15 points, which is regarded as an important difference (see above). Furthermore, statistical testing showed that the difference in this study population was significant (Wilcoxon signed rank test, p<0.001). Verbal IQ was higher than PIQ in all but 3 (VH, TO, SD) of these 25 children. Six (24%) of the 25 children had a difference of >=30 IQ points, 12 children (48%) had a difference between >=20 and <30 IQ points and 7 children (28%) had a difference between >=15 and <20 points between VIQ and PIQ.

6.2.1.3 *Performance IQ and Verbal IQ and associations with neurological status and motor function*

Figure 6.5 shows FSIQ, PIQ and VIQ according to neurological status at the age of 9-13 years. There was a significant association between neurological status and PIQ (Kruskal-Wallis test, p=0.004) but not between neurological status and VIQ (Kruskal-Wallis test, p=0.5)

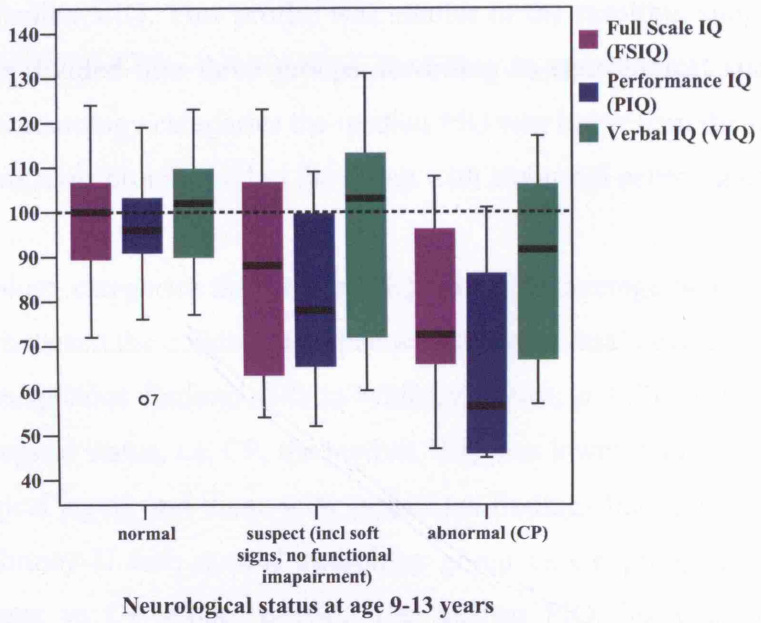


Figure 6.2: Full Scale IQ (FSIQ), Performance IQ (PIQ) and Verbal IQ (VIQ) on the WISC-R according to neurological status at time of this study. Displayed is the median (solid black horizontal lines), minimum and maximum (whiskers) and interquartile range (25%-75%) represented by the boxes. IQ scores are displayed on the Y-axis and categories of neurological status are displayed on the X-axis. The dotted black horizontal line displays the mean/median of 100 for a normative sample. O7 refers to an outlier (Subject BBe, PIQ 58).

Fifteen of the 44 children (34%) who were included in the analysis had normal neurological findings. Median FSIQ in this group was 100 (min 72-124), median PIQ was 96 (min 58, max 119) and median VIQ 102 (min 77, max 123). Nineteen of the 44 children (43%) had suspicious neurological findings on examination. In this subgroup, the median FSIQ was 88

(min 54, max 123), the PIQ 78 (min 52, max 109), and the VIQ 103 (min 60, max 131). Ten (24%) children were diagnosed with cerebral palsy (CP) on neurological examination. For this subgroup the median FSIQ was 73 (min 47, max 108), the PIQ 57 (min 45, max 101) and the VIQ 92 (min 59, max 119). Within the group of children with CP it was not possible to identify a pattern or associations between CP subtype and performance on psychometric testing. This is most likely due to the very small numbers in the CP subtype categories (between one and three cases in each CP subtype category).

As described above, in the whole group, the IQ profile was uneven, with the median PIQ lower than the median VIQ. This profile was similar in the resulting subgroups when the whole group was divided into three groups according to neurological status at age 9-13 years. In all three neurology categories the median PIQ was lower than the median VIQ and this difference was most pronounced in the group with abnormal neurological status.

In all three neurology categories the median VIQ was in the average range with only a very small difference between the children who had an entirely normal neurological examination and those with suspicious findings (Mann-Whitney U test, $p=0.7$). In the subgroup with abnormal neurological status, i.e. CP, the median VIQ was lower than in the subgroup with normal neurological status and those with suspicious findings but still within the average range (Mann-Whitney U test, normal neurology group vs CP group, $p=0.2$; group with suspicious findings vs CP group, $p=0.4$). The median PIQ, however, differed clearly between the three subgroups with the median PIQ in the normal neurology group being in the average range, and in the group with suspicious findings and the group with abnormal findings (CP) being in the borderline range and the below borderline range respectively (Mann-Whitney U test, normal neurology group vs suspicious neurology group, $p=0.05$; suspicious neurology vs CP group, $p=0.05$; normal neurology group vs CP group, $p=0.002$).

Performance on the TOMI provides a measure of motor impairment. Therefore, in addition to exploring the PIQ and VIQ within neurology categories, the relationship between TOMI error scores and PIQ and VIQ respectively was also investigated.

For the whole group, the TOMI scores showed a significant correlation with PIQ (Spearman's ρ= -0.5, p=0.001) but not with VIQ (Spearman's ρ = -0.2, p=0.3). The subtests of the performance scale all showed significant correlation with TOMI error scores (Spearman Rank Test; picture arrangement ρ= -0.4, p=0.002; block design, ρ= -0.5, p<0.001; object assembly, ρ= -0.4, p= 0.01; coding, ρ= -0.5, p=0.002) except picture completion for which there was only weak evidence of a correlation (ρ= -0.3, p=0.07). Picture completion is the only subtest of the PIQ scale that is "motor free"; the other subtests require the use of the hands to, for example, moving of picture cards, constructing blocks, using a pencil. In addition, visuo-motor co-ordination and working speed are required for these subtests.

When only children with normal neurological status were included in the analysis, no significant correlation between TOMI scores and any of the PIQ subtests remained. Neither were there any significant correlations when only the data of the subgroup with suspicious neurological status were investigated. These findings and the associations between neurological status and IQ scales presented above, indicate that the PIQ in this population is determined in great part by the presence and severity of neuromotor impairment.

6.2.1.4 Performance IQ and Verbal IQ according to ultrasound categories

There was no significant association between ultrasound categories (normal, non-parenchymal lesion, parenchymal lesion) and VIQ, and only weak evidence for an association with PIQ scores (Kruskal-Wallis test; VIQ, p=0.3; PIQ, p=0.09).

Interestingly, the median scores in the group with normal neonatal ultrasound and those with non-parenchymal lesions were similar (normal ultrasound: median FSIQ 91; min 85, max 123; PIQ 92; min 78, max 103; VIQ 102, min 77, max 131. Non-parenchymal lesions: median FSIQ 96; min 60, max 124; PIQ 94; min 45, max 119; VIQ 102; min 72, max 123). In the group with parenchymal lesions, the median FSIQ was 74 (min 47, max 116), PIQ 69 (min 45, max 109) and median VIQ 91 (min 57, max 119).

6.3 Cognitive function and epilepsy

6.3.1 Comparison of Performance IQ and Verbal IQ between the group with epilepsy and the group without epilepsy

Of the 44 children included in the analyses, 15 (34%) had epilepsy and 29 (66%) did not have epilepsy. There were 5 girls and 10 boys in the group with epilepsy, and 15 girls and 14 boys in the group without epilepsy.

Figure 6.3 shows FSIQ, PIQ and VIQ for the group with and the group without epilepsy. In the group with epilepsy, median FSIQ was 79 (min 47, max 124), median PIQ 70 (min 45, max 119) and median VIQ 80 (min 57, max 122). In the group without epilepsy, median FSIQ was 98 (min 54, max 123), median PIQ 96 (min 54, max 118) and median VIQ 102 (min 60, max 131). PIQ was significantly lower in the group with epilepsy (Mann-Whitney U test, p=0.03). Although the there was a large difference between the epilepsy and the non-epilepsy group in median VIQ, there was only weak evidence on statistical testing for a significant difference (Mann-Whitney U test, p=0.09). This result on statistical testing might be due to the relatively small group sizes. The IQ profile was similar in the group with and the group without epilepsy with the PIQ being lower than the VIQ.

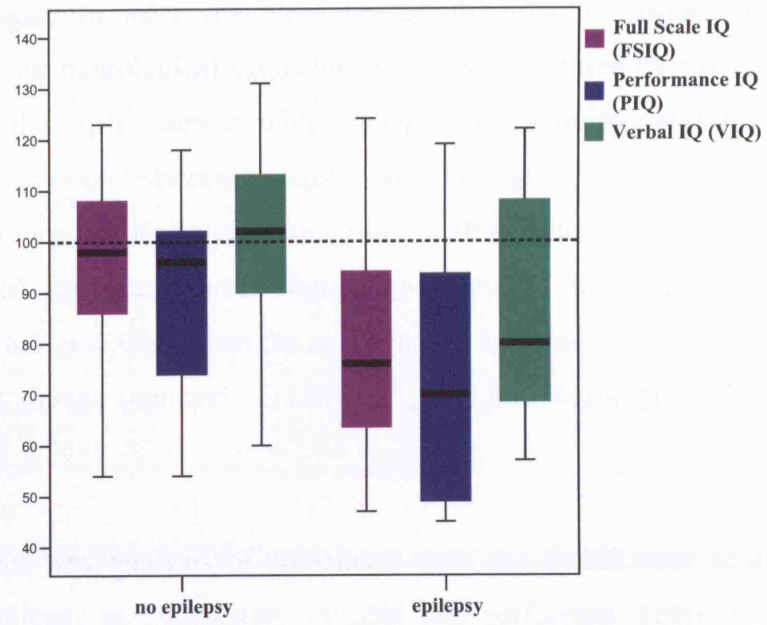


Figure 6.3: Full Scale IQ (FSIQ), Performance IQ (PIQ) and Verbal IQ (VIQ) on the WISC-R for the group of children with epilepsy (n= 15) and the group of children without epilepsy (n=29). Displayed is the median (solid black horizontal lines), minimum, maximum (whiskers), and interquartile range (25%-75%) indicated by the boxes. IQ scores are displayed on the Y-axis. The dotted black horizontal line displays the mean/median of 100 for a normative sample.

6.3.2 Effect of epilepsy on Performance IQ and Verbal IQ with adjustment for neurological status and motor function

There was a significant difference between the epilepsy and the non-epilepsy group in the proportion of children with abnormal neurological status (6/15, of those with epilepsy, i.e. 40%, had CP compared with 4/29, i.e. 14%, of those in the group without epilepsy; Chi Square test, linear by linear association, p=0.05). In addition, there was a significant difference of severity of motor impairment as indicated by the TOMI scores (Mann-Whitney-U test, p=0.01) between the group with and the group without epilepsy.

Since neurological status in the whole group showed a significant association with PIQ, the significant difference in PIQ between the epilepsy group and non-epilepsy group might be associated with abnormal neurological status and impaired motor skills rather than the

presence of epilepsy. In order to explore whether the effect of epilepsy on PIQ remained once adjustment for neurological status has been made, a (three by two) factorial analysis of variance with the explanatory variables epilepsy and neurological status was performed. There was no interaction between epilepsy and neurological status. There was no longer a significant difference in PIQ between the group with and the group without epilepsy after having accounted for neurological status (epilepsy: $F(1/40)=1.3$, $p=0.25$, neurological status: $F(2/40)= 6.1$, $p=0.05$). When the analysis was repeated for VIQ, the association with epilepsy did not change (epilepsy: $F(1/40)=3.3$, $p=0.08$, neurological status: $F(2/40)=0.4$, $p=0.7$).

In addition, a one-way analysis of covariance with the TOMI error scores entered as a covariate and epilepsy as explanatory variable was performed. There was no interaction between TOMI scores and epilepsy. Once performance on the TOMI, a measure of impairment of motor function, was taken into account, the association between epilepsy and PIQ was no longer significant (epilepsy: $F(1/41)=0.78$, $p=0.38$; TOMI: $F(1/41)=10.3$, $p=0.003$). When the analysis was repeated for VIQ the association with epilepsy did not change (epilepsy: $F(1/41)=3.4$, $p=0.07$, TOMI: $F(1/41)=0.2$, $p=0.7$).

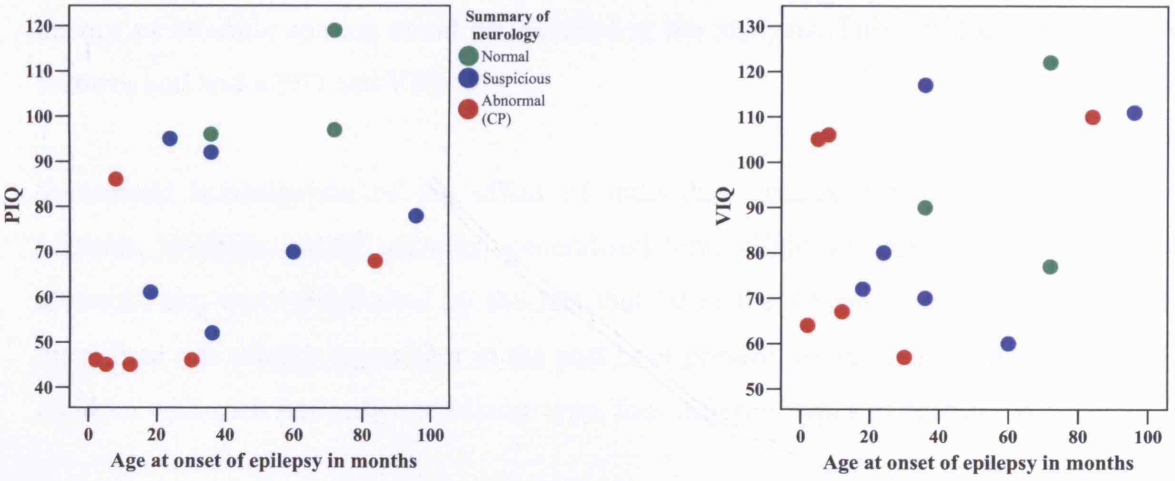
It should be noted that the analyses described in this section are not non-parametric tests. However, since the nature of these additional analyses was of mainly explorative nature, it was felt that it was appropriate to apply these parametric tests in this context.

6.3.3 Associations between cognitive function and age at onset of epilepsy, seizure frequency and seizure type

Information on age at epilepsy onset, seizure frequency, and seizure type was available for all 15 children with epilepsy who were included into the analyses.

6.3.3.1 *Age at onset of epilepsy and cognitive function*

Figure 6.4 a and figure 6.4 b below show the relationship of age at onset of epilepsy with PIQ and VIQ respectively. On statistical testing there was no evidence that age at epilepsy onset was associated with VIQ (Spearman's ρ=0.39, p=0.14). There was, however, a significant association with PIQ (Spearman's ρ=0.5, p=0.03). On inspection of the colour coded scatter plots there appears to be an association between PIQ, age at onset of epilepsy and neurological status, in that those with abnormal neurological status had a low IQ and early onset epilepsy. For VIQ this pattern seemed less obvious.



Figures 6.4 a and b: Scatter plots showing the relationship between age at onset of epilepsy and Performance IQ (a) and Verbal IQ (b) the children with epilepsy (n=15). The colour coding indicates the neurological status at the time of this study (age 9-13 years) for each subject.

6.3.3.2 *Seizure frequency and cognitive function*

There was no significant association between the IQ scores and seizure frequency (Spearman Rank test; PIQ: ρ=0.2; p=0.5; VIQ: ρ=0.4, p=0.2).

6.3.3.3 Seizure type and cognitive function

Of the 15 children with epilepsy who were included in the analysis, 1/15 had only focal seizures, 6/15 had focal and generalised seizures and 8/15 had generalised seizures only (i.e. no focal onset witnessed). There was no association between seizure types as categorised above and IQ scores (Kruskal-Wallis test; PIQ, p=0.3; VIQ, p=0.6), nor was there an association between the presence/absence of more than one seizure type and IQ scales (Mann-Whitney U test; PIQ, p=0.6; VIQ, p=0.5).

Only three of the eight children with a history of neonatal seizures were able to complete the psychometric testing (BK, AM, WS). PIQ and VIQ were <=70 in all cases with the exception that one child (WS) had a VIQ of 105. Only one (AM) of the four children with a history of infantile spasms could be included in the analysis. This child also had neonatal seizures and had a PIQ and VIQ <70.

Systematic investigation of the effect of individual seizure types, e.g. simple partial seizures, complex partial seizures, generalised tonic-clinic seizures, simple or complex absences etc, was complicated by the fact that 10 of the 15 children in this analysis had more than one seizure type either in the past or at present. In addition, in the group of five children who each had only one seizure type, four different types of seizures were seen.

6.4 Associations between clinical variables and cognitive outcome

The aim of this section is to investigate, in the whole group, the associations between clinical variables that are routinely collected in a clinical setting and cognitive outcome in an attempt to identify useful clinical factors for prediction of cognitive outcome in this study population. Some aspects regarding relationships between cognitive outcome and the clinical variables of neurological status, performance on testing of neuromotor function (TOMI) and epilepsy have already been presented above (see sections 6.2.1.3, 6.3.1, 6.3.2). Univariate analyses were performed to explore associations of individual clinical variables

with PIQ and VIQ at age eight years. In a second step regression analyses were performed to determine whether a single variable or a set of clinical variables provide useful prognostic information for cognitive outcome. In a third step, regression analyses including clinical and MR imaging variables were performed, and these data are presented and discussed in chapter 10. For the analyses described in this section, data were included from the 44 children who had complete assessments including psychometric testing.

6.4.1 Selection of variables for investigation

Based on the existing literature and on clinical observations in the population of the current study, the following variables were selected for examination: Gender, gestational age at birth, birth weight, APGAR score at 5 minutes (categories for the variables gestational age, birth weight, APGAR score at 5 minutes: see legend to table 6.2), duration of oxygen supplementation (as a surrogate measure of chronic lung disease if >37w GA), presence or absence of neonatal seizures, neurological status at term (normal, equivocal, abnormal), neonatal ultrasound findings (normal, non-parenchymal, parenchymal lesion), persistent ductus arteriosus (PDA; requiring either surgical or drug treatment) neurological status at the time of the current study (normal, suspicious, abnormal) and TOMI scores as a measure of motor function and presence or absence of epilepsy. There was no case with intracranial infection (meningitis, encephalitis) in this group. Information on socio-economic status and maternal education had not been recorded sufficiently for all participants. Therefore, these two variables could not be included in the analysis.

6.4.2 Results of the univariate analyses

Table 6.2 below shows the results of the univariate analyses investigating the associations of the selected clinical variables with PIQ and VIQ.

Table 6.2: Associations between clinical variables and Performance and Verbal IQ for the whole group (n=44)

	Performance IQ	**Verbal IQ**
Gestational age, weeks *,***	p=0.76	p=0.14
Birth weight, g *, ***	p=0.05	p=0.04
Gender **	p=0.7	p=0.1
APGAR at 5 minutes ** (<=5 or >5)	p=0.6	p=0.9
Duration of oxygen supplementation (<= or > 37 w GA) **	p=0.008	p=0.002
PDA[#] **	p=0.07	p=0.07
Neonatal seizures **	p=0.02	p=0.12
Neonatal ultrasound findings ***	p=0.09	p=0.3
Neurological status at term ***	p=0.3	p=0.5
Neurological status at time of current study ***	p=0.04	p=0.5
TOMI error score ****	ρ= -0.5; p=0.001	ρ= -1.8; p=0.3
Epilepsy **	p=0.03	p=0.09

* Categories for BW :<500g, 500 - <750g, 750 - <1000g, 1000g - <1500g, >=1500g
Categories for GA: <28 wGA, 28 - <=32 wGA, >32 wGA
** Mann Whitney U test
*** Kruskal Wallis test
**** Spearman Rank test
[#] PDA= persistent ductus arteriosus

A significant association for both PIQ and VIQ was detected for the variable "duration of oxygen supplementation" (i.e. need for oxygen supplementation beyond 37 weeks GA) and for birth weight. There was weak evidence on statistical testing that birth weight and the presence of PDA were associated with both PIQ and VIQ.

For VIQ, in addition to duration of oxygen supplementation >37 w GA, birth weight and PDA, there was weak evidence for an association with epilepsy.

For PIQ, in addition to duration of oxygen supplementation >37 w GA, birth weight and PDA, there was also a significant association with presence of epilepsy. Neurological status at the time of this current study and neuromotor function as indicated by the TOMI scores

were significantly associated with PIQ (this has been explored in more detail and presented in section 6.2.1.3 above). There was only weak evidence for an association between neonatal ultrasound diagnosis (categorised as described above) and PIQ. The presence of neonatal seizures showed a significant association with PIQ. However, there were only three cases with neonatal seizures in the whole group so this statistical result has to be treated with caution.

6.4.3 Regression analyses

The regression analyses presented in this section were performed within the General Linear Model and the overall approach regarding the statistical analyses employed in this study is discussed in chapter 4, section 4.5.

Transformation into categorical variables was performed for the continuous variables birth weight, gestational age (categories for birth weight and gestational age, see legend to table 6.2) and APGAR score at 5 minutes (category "below 5" and category "over 5") so that assumptions for regression analyses were met by accounting for a possible non-linear relationship with the outcome variables. Next, correlation matrices were created to identify correlations between independent perinatal and neonatal variables. Based on these correlation matrices, principal component scores (PCAs) were calculated for the correlated independent perinatal and neonatal variables gestational age, birth weight, ultrasound category, APGAR at 5 minutes, duration of oxygen supplementation and persistent ductus arteriosus (PDA) to obtain a reduced dataset of "derived" explanatory variables that are orthogonal (not correlated with each other), thus dealing with the problem of multi-collinearity. The first two sets of derived variables, which contributed most to explanation of the variance of the dependent variables, namely "PCA_1" (mainly dominated by birth weight, gestational age and by duration of oxygen supplementation) and "PCA_2" (dominated by ultrasound diagnosis and secondly PDA) from this reduced dataset were entered in the regression model and additional variables of interest were added to the model (gender, epilepsy, neurological status at term age, neonatal seizures, TOMI error scores).

Neurological status at time of this study was not included since, as expected, it was highly correlated with the TOMI scores (Kruskal-Wallis test, $p<0.001$) and showed a similar relationship with the IQ scales and the subtests as it had with the TOMI scores. Therefore, TOMI scores were included under the assumption that they give a measure of the severity of motor impairment and can also serve as a surrogate variable for neurological status.

Variables were entered one at a time and the contribution of each variable to the explanation of the variance of the outcome variable (PIQ or VIQ respectively) was assessed at each step. As outlined in chapter 4, section 4.5, the regression analyses are focused on the question whether a single variable or a set of variables provide clinically useful prognostic information rather than trying to quantify risk or infer causation.

6.4.3.1 Results of the regression analyses

6.4.3.1.1 Performance IQ

Table 6.3 below shows the results of the final model that retained PCA_1 (mainly dominated by birth weight, gestational age and by duration of oxygen supplementation) and TOMI score as the variables that independently contributed significantly to the prediction of PIQ.

PCA_2 (mainly dominated by ultrasound category and secondly PDA), gender, neurology at term age and neonatal seizures did not contribute independently in a significant way to the explanation of the variation of PIQ. Without TOMI score in the model there was weak evidence that epilepsy was independently related to PIQ ($p=0.06$). This effect, however, disappeared (epilepsy $p=0.48$) when the TOMI scores were entered into the model (TOMI $p=0.03$ with epilepsy in the model).This pattern remained similar once gender, neurological status at term, PCA_2 and neonatal seizures had been removed from the model and only PCA_1, TOMI and epilepsy were retained.

Table 6.3: Results of the regression analysis with PIQ as the dependent variable; final model

	B	Standard error of B	t-value	p - value
Constant	94.1	4.2	22.3	0.000
PCA_1*	3.5	1.6	2.2	0.03
TOMI	-2.1	0.6	-3.7	0.001

*PCA scores = derived scores calculated from: gestational age, birth weight, APGAR5, PDA, duration of oxygen supplementation, ultrasound category; PCA_1 determined by birth weight, gestational age and by duration of oxygen supplementation.
B= unstandardised regression coefficient

6.4.3.1.2 Verbal IQ

The only variable that contributed independently to prediction of VIQ was PCA_1, which is mainly dominated by birth weight, gestational age and by duration of oxygen supplementation (see table 6.4).

Neither PCA_2 (mainly dominated by ultrasound category and secondly PDA) nor gender, neurological status at term, neonatal seizures, TOMI scores nor epilepsy contributed independently in a significant way to prediction of VIQ when added to the model. Without TOMI score in the model there was weak evidence that epilepsy contributed to the prediction of VIQ (p=0.08). This effect disappeared once TOMI score was added to the model.

Table 6.4: Results of the regression analysis with VIQ as the dependent variable; final model

	B	Standard error of B	t-value	p - value
Constant	95.8	2.8	36.2	0.000
PCA_1*	5.0	1.5	3.3	0.002

* PCA scores = derived scores calculated from: gestational age, birth weight, APGAR5, PDA, duration of oxygen supplementation, ultrasound category; PCA_1 determined by birth weight, gestational age and by duration of oxygen supplementation.
B= unstandardised regression coefficient

6.4.4 Summary of analyses investigating associations between clinical variables and cognitive function

The univariate analyses performed on the whole group showed significant associations for both PIQ and VIQ with oxygen supplementation beyond 37 w GA and weak evidence for an association with birth weight and with the occurrence of a PDA. Statistical testing revealed a significant association between the presence of epilepsy and PIQ, and indicated a weak association for epilepsy and VIQ. In addition, for PIQ, a significant association was identified with the occurrence of neonatal seizures and neurological status at time of the current study, and with the TOMI error scores, and there was weak evidence for an association with neonatal ultrasound diagnosis.

The subsequent regression analyses identified the TOMI score and one of the PCA scores derived from perinatal and neonatal variables, i.e. PCA_1, which is dominated by gestational age, birth weight and duration of oxygen supplementation, as the best independent clinical predictors of PIQ. The best independent clinical predictor for VIQ was PCA_1.

Associations between PIQ or VIQ and neurological status at the time of this current study, TOMI scores and epilepsy were explored further in a more detailed and partly descriptive way (see sections 6.2.1.3, 6.3.1 and 6.3.2). It appeared that in the whole group, PIQ was heavily influenced by the absence/presence of motor impairment in contrast to VIQ, which was fairly stable across the different neurology categories and TOMI error score scales. In the subgroup with epilepsy, the proportion of children with motor impairment was higher than in the subgroup without epilepsy, once adjustment had been made for motor impairment, the difference in PIQ between those with epilepsy and those without epilepsy was no longer significant.

Univariate analyses had identified a weak association between neonatal ultrasound diagnosis and PIQ. The results of the regression analyses, however, indicated that cranial

ultrasound diagnosis (contained in the variable PCA_2) was not a good independent predictor for PIQ.

6.5 Discussion

6.5.1 Cognitive outcome and IQ profile in the whole group

Although the sample in this current study is not a random but a selected sample, the overall cognitive outcome of the study population as indicated by the IQ scores on the Wechsler Scale is similar to the outcomes reported previously in the literature for both hospital based and population based studies, with median FSIQ, VIQ and PIQ in the normal range but below the mean of a typical population (Saigal et al, 1991; The Scottish Low Birthweight Study Group, 1992; Botting et al, 1998; Wolke and Meyer, 1999; Saigal, 2000; Bhutta et al, 2002).

Many of the existing studies differ in their inclusion criteria, e.g. regarding inclusion or exclusion of children with neurological impairment. Some studies included all surviving preterm children and some of those studies assigned the lower limit of IQ scores to children that had severe neurological impairments that prevented them from completing the psychometric tests (e.g. Roth et al, 1993, 1994). Other studies report only on children who attend mainstream education or on those without severe neurological impairments (e.g. Botting et al, 1998). Nevertheless, the overall pattern in the majority of the existing studies is similar with overall lower scores on psychometric testing in the preterm children when compared to controls, with a mean difference of 10 IQ points or more. In addition, the difference to controls remains, although less pronounced, once children with neurological impairments have been excluded. For example, Saigal et al (2000) found that when children with neurosensory impairments and an FSIQ <85 were excluded from the analysis, the mean IQ increased by 10 points but was still 5 points lower than in controls. In the current study children with neurological impairments so severe that they prevented the children

from completing the WISC-R were excluded from the analysis. However, a considerable proportion of children included in the analysis had abnormal neuromotor findings; with 10/44 children having been diagnosed with CP (see section 6.2.1.3). The inclusion of children with neurological impairments might partly explain the wide variation seen in both PIQ and VIQ scale in the current study.

Regarding the IQ profile, irrespectively of FSIQ, there was a difference of >=15 IQ points between PIQ and VIQ in over half of the children. In all but three of these children VIQ was higher than PIQ. This pattern has been described previously and is seen even when children with neurological and neurosensory impairments had been excluded as, for example, in the population based study conducted by Botting et al (1998).

When the whole group was divided according to neurological status, the pattern in the three neurology categories regarding differences between PIQ and VIQ remained, similar to that in the whole group. However, the difference between the two IQ scales was less pronounced in those with normal neurology compared to the children with suspicious or clearly abnormal neuromotor findings. Interestingly, in contrast to PIQ, median VIQ was not significantly associated with neurological status or performance on the TOMI. This indicates that motor impairment subsequent to damage to periventricular white matter tracts is likely to play a considerable part in how the children perform on the PIQ scales. The finding that all subtests of the PIQ scale, except picture completion, which can be regarded as a "motor free" test, were significantly associated with the degree of motor impairment as indicated by the TOMI, supports this. It is of note that children in the group with suspicious neurological status, i.e. unspecific neurological findings but no functional impairment, had lower median PIQ scores than those with normal neuromotor findings. This might indicate that in this subgroup subtle focal lesions to white matter tracts or possibly diffuse brain lesions are present that are not sufficiently severe to cause major neuromotor impairment but affect areas that are involved in the control of perceptual-motor and/or spatial skills, i.e. skills that, like working speed and fine motor co-ordination, are required for the PIQ subtests.

6.5.2 Gender

No significant gender differences were detected in IQ scores, which is in keeping with findings in the existing literature (Saigal et al 1991; Horwood, Mordrige and Darlow, 1998), although there are also reports that suggest poorer overall and/or cognitive outcome in boys compared to girls (Marlow et al, 2005; Taylor et al, 2000). Although not significantly different, the median scores on both the VIQ and the PIQ were higher in males than in females. The difference in PIQ might be partly explained by the fact that there was a slightly higher percentage of girls than boys with cerebral palsy, which might affect performance on most PIQ subtests. There was, however, no evidence for a difference in TOMI error scores between boys and girls (Mann-Whitney U test, p= 0.7). In addition, the slightly higher percentage of girls than boys with CP is unlikely to explain the (non-significant) differences in VIQ scores.

6.5.3 Performance and Verbal IQ according to ultrasound categories

There was no strong evidence on statistical testing for an association between the neonatal cranial ultrasound categories (normal, non-parenchymal lesion, parenchymal lesion) in this study population and IQ scores of the children whose data could be included in the analysis. However, for PIQ there was weak evidence that neonatal ultrasound category and performance on the subtests of the PIQ scale were associated. Median IQ scores were similar in the categories "normal" and "non-parenchymal" lesions, with median scores on both IQ scales in the normal range. In contrast, median IQ scores in the category "parenchymal lesion" were lower, with the PIQ most affected. There have been previous studies investigating the relationship between neonatal cranial ultrasound findings and outcome at school age and, although most studies looked at FSIQ and not at VIQ and PIQ separately, it has been shown that parenchymal lesions in particular are associated with poor cognitive outcome (e.g. Roth et al, 1993, 1994). Since in the population in the current study differences in FSIQ were mainly due to lower PIQ scores, the findings of this study and the reports in the literature are not inconsistent. However, it has also been shown that

neonatal ultrasound has limited predictive value for long term cognitive outcome, in particular, for those who have no parenchymal lesions. In addition, the sample of this current study was not a random sample, e.g. there were only six children with normal ultrasound, making a comparison between those with normal and those with an abnormality on ultrasound difficult. However, non-parenchymal and parenchymal lesions were approximately equally distributed making a comparison between these two categories possible and the results obtained from the comparison of these two categories are consistent with those of numerous previous studies (see above and chapter 3, section 3.3.2 and section 3.4). The associations between cognitive function and brain lesions are discussed in more detail in chapter 10. which deals with the results of analyses investigating associations between visual and morphometric assessments of MRI data collected in this study population at the age of 9-13 years and cognitive outcome.

6.5.4 Cognitive function and epilepsy

While there is only limited information available in the existing literature dealing specifically with preterm children with epilepsy, there are numerous reports that show that childhood epilepsy can be associated with a spectrum of problems, including cognitive impairment (Nolan et al 2003; O`Leary, Burns and Borden; 2006), behavioural problems (Davies, Heyman and Goodman, 2003) and impaired quality of life, and a range of individual risk factors have been described. It has to be kept in mind that, since childhood epilepsy is a heterogeneous disorder, the reported findings with regards to overall cognitive function are heavily influenced by the samples that are chosen to be studied (Bourgeois et al, 1983). Based on a review of the literature, Noecker, Haverkamp-Krois and Haverkamp (2005) have suggested a conceptual framework of mental health status in childhood epilepsy that attempts to integrate the mode of action of the identified individual risk factors. This framework consists of three levels of investigation: pathogenetic causes (underlying brain pathology, age at onset of epilepsy, type of epilepsy), mediators and moderators connecting causes to outcomes (including e.g. effects of antiepileptic drugs,

seizure activity), mental health outcome (risk for learning disability, psychopathology, impaired quality of life).

The current study focuses on overall cognitive functions as assessed with the Wechsler Scale and on a selected group of preterm children. In addition, in this particular group of children there is a high frequency of structural brain abnormalities (this is discussed in more detail in chapters 7 and 8). However, although this study deals with a selected group, the findings described here are consistent with reports in the existing literature on childhood epilepsy and associations with cognitive function (Bailet and Turk, 2000; Nolan et al, 2003; Aldenkamp et al, 2005; O`Leary, Burns and Borden, 2006). In the current study, median scores for PIQ and VIQ were lower in the group of children with epilepsy when compared to those without epilepsy. The differences in median IQ scores were significant for PIQ on statistical testing. For VIQ, the results of statistical testing indicated weak evidence for those with epilepsy performing worse on these scales. Hoie et al (2005), in a population based study, reported a high frequency of non-verbal problems in children with epilepsy. These problems were particularly frequent in children with remote symptomatic epilepsies (defined by the authors as epilepsy with an obvious etiological factor that might be regarded as responsible for the brain dysfunction such as cerebral malformation, intracranial haemorrhage, intracranial infection, serious perinatal complications, traumatic brain injury, cranial tumours, progressive encephalopathy) and in children with therapy resistant seizures. In contrast, in children with idiopathic and localisation related idiopathic epilepsy syndromes, simple partial or absence seizures, a low frequency of non-verbal problems was seen. However, the study did not comment on the neurological status of the children.

In the current study, the significant difference in PIQ between those with and those without epilepsy were no longer seen once neurological status and/or severity of neuromotor impairment was accounted for. Ellenberg, Hirtz and Nelson (1986) have investigated FSIQ in children with afebrile seizures and found that cognitive impairment was more common in those with epilepsy, but they found also that this was accounted for by children who had neurological abnormalities before their first seizure. Steffenburg et al (1995) found in a

population-based study in children with mental retardation (defined as an FSIQ <70) and active epilepsy that 70% had at least one additional neurological impairment such as cerebral palsy and visual impairment. The results of this current study and those of several studies reported in the literature suggest that there is a large effect of neurological impairment on PIQ and FSIQ and that there is no major independent effect of epilepsy in the populations studied. It is very likely that there is a dominant impact of underlying brain lesions and this will be investigated in more detail in subsequent chapters.

Interestingly, but not unexpectedly, the (non-significant) difference in VIQ between the group with and the group without epilepsy remained unchanged when neuromotor impairment was accounted for in the analysis. Toulopoulou et al (2004), in a family study on schizophrenia, found that in patients, unaffected relatives, and controls, volume of the hippocampi correlated with VIQ in all three groups and, in addition, in unaffected relatives there was also an association of left hippocampal volume with PIQ. Hippocampal abnormalities have been described in preterm populations (e.g. Isaacs et al, 2000; Nosarti et al, 2002) and hippocampal abnormalities are a frequent finding in patients with epilepsy (Dam, 1980; Bernasconi et al, 2003). It might therefore be possible that in this current study the difference in VIQ may partly be explained by a higher frequency of hippocampal abnormalities in the group with epilepsy. This question is addressed in more detail in subsequent chapters that deal with the neuroimaging findings in the population of the current study.

Age at onset of epilepsy showed a significant association with PIQ but not with VIQ. Given that there was a significant association between neurological status and age at onset of epilepsy (see chapter 5) and also between PIQ and neurological status (discussed above), it is very likely that the association between age at onset of epilepsy and PIQ is influenced by neurological status, which in turn is likely to be associated with the presence and extent of brain injury.

It has to be kept in mind that the current study investigates a very selected sample of children with epilepsy, with the assumption that epilepsy is caused by an underlying early

acquired brain lesion of fairly homogenous etiology. Therefore, existing studies that examine the effects of age at onset of epilepsy on cognitive outcome (some of which are outlined below) and include heterogeneous groups of children with epilepsy may not be directly comparable to the findings in this study.

There are somewhat inconsistent findings in the existing literature regarding associations between age at onset and long term cognitive outcome in childhood epilepsy, and this may partly be due to the fact that the most detailed information is available mainly for some syndromes such as infantile spasms and sparse for seizures that are not part of recognized syndromes. However, it has been suggested that a poor prognosis of early onset epilepsy is largely due to the presence and extent of causal brain lesions. Hoie et al (2005), in their population based study, found that onset of epilepsy before the age of 10 years was associated with a significant risk of non-verbal problems and that the risk was highest when the onset was before two years of age. Nolan et al (2003) investigated associations between FSIQ and age at onset in different epilepsy syndromes in a hospital based study, and identified age at onset as a significant factor predicting cognitive outcome with age at onset varying between the different epilepsy syndrome groups that were examined. O`Leary, Burns and Borden (2006) included only children with an FSIQ, PIQ or VIQ of >=70 in an analysis comparing children with different types of epilepsy with age and gender matched controls. No significant association between IQ scores and age at onset of seizures was found in this study. However, in contrast to the studies mentioned above, age was categorised only into two categories, below and above six years, and the sample was relatively small (n=27). However, all three studies (Nolan et al, 2003; Hoie et al, 2005; O`Leary, Burns and Borden, 2006) did not comment on the possible relationship between age at onset and an underlying structural brain abnormality. Chevrie and Aicardi (1978), in a hospital based study, found that the majority of children with onset of epilepsy in the first year of life had mental retardation at age five years. In this series the type of seizures had an influence on outcome, with children who suffered from infantile spasms having worse cognitive outcome than those with other seizure types. Similar results are reported by Cavazzutti et al (1984). In the current study only one child with a history of infantile spasms was able to complete the psychometric assessment and the results indicated poor

cognitive function. In the other three children the neuromotor impairment was so severe that they were unable to complete the psychometric testing.

The eight children in this study with neonatal seizures had a poor outcome regarding neurological status. All except one child had developed CP and only three were able to complete the cognitive assessment; the children who had complete cognitive testing generally scored poorly on the IQ scales (PIQ showed a significant association with the presence of neonatal seizures) except one child who had a VIQ in the average range. This was the only child with non-parenchymal lesions on neonatal cranial ultrasound; all other children with neonatal seizures had been diagnosed with parenchymal lesions.

The finding that seizure frequency was not associated with cognitive outcome is not in accordance with most reports in the literature, which indicate that the frequency of seizures has a negative impact on cognitive function (Bourgeois et al 1983; Farwell, Dodrill and Batzel, 1985; Bulteau et al, 2000). However, many existing studies focus on children with difficult to treat or intractable epilepsy. In the current study there were only very few children with frequent seizures. This might partly explain the difference in the findings in the current study compared to previous literature.

No significant relationship between seizure type (seizures categorised as focal or generalised) and cognitive outcome was found. A more detailed systematic investigation of the relationship between individual seizure types (e.g. simple partial, complex partial, generalised tonic-clonic, tonic, myoclonic, simple or complex absences etc) was complicated by the fact that many of the children had more than one seizure type. In addition, it has to be kept in mind that seizures were classified on the basis of seizure semiology via description of seizures obtained from the parents and that the interictal surface EEG recordings did not improve classification of seizures substantially. Therefore, the findings in this current study remain somewhat inconclusive regarding the relationship between seizure type and effect on cognitive outcome.

The analyses discussed above and in the next section were performed on data available from 44 children of the original group of 54 children. Ten of the 54 children had to be excluded from the analyses. Eight of these 10 children were unable to complete the WISC-R since they had severe neuromotor impairments preventing them from completing the required number of subtest that are necessary for computation of IQ scores and for 2 of the 10 children no testing with the WISC-R was done at the 8 year follow-up. All but one of the excluded children had epilepsy. It is possible that the exclusion of these children, the majority of whom had severe neuromotor impairment, might have caused a bias when comparing the overall cognitive outcome between those with and those without epilepsy in that the difference in cognitive function between the two groups might be underestimated. The group of excluded children did not differ significantly in perinatal and neonatal variables except in the proportion of emergency caesarean section and neonatal seizures. There was also a difference in the frequency of lesions on ultrasound ($p=0.05$ on statistical testing). Some of the analyses were repeated with the eight children who had not been able to complete the WISC-R included. In these analyses, minimum scores of 45 for PIQ and VIQ were assigned to the children who did not complete the assessments due to their severe motor impairments (the two children who did not have a psychometric assessment at all were still excluded from the analyses).The difference between those with and those without epilepsy was more pronounced when the eight children with a minimum score assigned were included. The identified patterns of associations were similar when analyses were performed on the group with the 10 children excluded and the group with the 8 children included. It should be noted that the assignment of a minimum score is likely to bias the analysis in the opposite direction to that of the exclusion of the eight children. Since the subjects were excluded on the basis that their neurological impairments, not necessarily their intellectual impairments, made it impossible for them to perform the required tasks, the decision was made to perform the analyses described in this chapter on those study participants for whom data were available.

6.5.5 Statistical analysis investigating associations between clinical variables and cognitive outcome

A summary of the univariate analyses and regression analyses investigating associations between clinical variables and cognitive function has been given above in section 6.4.4. and some more detailed aspects of the relationships between neurological status, epilepsy, gender and IQ scores have been discussed above in section 6.5.1, 6.5.2 and 6.5.4.

The study population of the current study is not a random sample and this has to be kept in mind when discussing the associations between clinical variables and outcome and the contribution of clinical variables to prediction of outcome. Nevertheless, the findings obtained from the univariate analyses are consistent with numerous hospital and population based studies. Birth weight has been shown to be associated with overall cognitive outcome in many previous studies (for review, see Bhutta et al, 2002). The need for oxygen supplementation after 37 w GA showed a significant relationship with both PIQ and VIQ and this too is in accordance with the results of previous reports in the existing literature (for review, see Bhutta et al, 2002). The presence of PDA, for which there was weak evidence for an association with cognitive outcome (PIQ and VIQ) has been shown to be related to cognitive function (Cooke et al, 2005). It has been demonstrated that PDA and increased duration of oxygen duration is associated (Jobe and Banclarini, 2001) and also that PDA is associated with an increased risk for intracranial haemorrhage (Meek et al, 1999), which in turn is likely to affect developmental and cognitive outcome. For PIQ only, univariate analyses revealed associations with neurological status at the time of this study, as well as with TOMI error scores, and provided weak evidence for an association with neonatal cranial ultrasound findings, suggesting that there is some underlying brain damage to white matter tracts affecting visuo-perceptual and possibly executive functions.

Many studies have identified the impact of socio-demographic and environmental factors on cognitive outcome in preterm children (Hack et al, 1992; Taylor et al, 2004) and some studies (Fawer et al, 1995; Gross et al, 2001) have suggested that in those children who survive without brain lesions, these factors are better predictors for long term cognitive

outcome and performance at school than perinatal factors. It was not possible to include these factors in the analyses performed in the context of the current study since the relevant data had not been collected in sufficient detail.

In the regression analyses, the only clinical variables that were identified as contributing individually in a significant way to prediction of outcome in this group of preterm children were the TOMI scores for PIQ and the composite score (derived from the five perinatal variables gestational age, birth weight, APGAR at 5 minutes, presence of PDA, need for oxygen supplementation after 37 w GA and ultrasound category) that was dominated by birth weight, gestational age and duration of oxygen supplementation for both PIQ and VIQ. The latter finding, that the degree of immaturity is associated with cognitive outcome, is consistent with reports in the existing literature (e.g. Saigal et al, 1991; for review see Bhutta et al, 2002). This is likely to indicate the vulnerability of the immature brain, but also that superimposed on this vulnerability, factors associated with the severity of the illness (here reflected by the need for oxygen supplementation over a long time) are likely to have a negative impact on the developing brain. It has been shown previously that the presence and extent of brain injury is a strong factor in neurological and overall cognitive outcome and that there is an association between the degree of immaturity and frequency of brain lesions in preterm populations (see chapter 3). The finding that TOMI (i.e. the degree of neuromotor impairment) was an independent predictor for PIQ suggests that the presence and possibly the degree of periventricular lesions is an important factor determining outcome in this population. Visual and morphometric analysis of MRI data that allow more detailed investigation of associations between brain injury and outcome than neonatal ultrasound are part of this study and are discussed in subsequent chapters. Epilepsy did not independently contribute to prediction of PIQ or VIQ when entered with the other variables in the regression model. The results of the regression analyses and, in particular, the results for PIQ may indicate that the presence of epilepsy is more to be regarded as a marker of brain injury than an individual factor contributing independently to impaired outcome in this particular population of preterm children. This is investigated and discussed in more detail in chapter 10.

6.6 Conclusions

In the whole group, both median VIQ and median PIQ as assessed on the Wechsler Scale were within the normal range and the upper end of the low average range respectively. A wide variation in IQ scores was seen for both scales. The IQ profile was uneven with the PIQ being lower than the VIQ, irrespectively of the presence or absence of epilepsy. The difference between VIQ and PIQ was more pronounced in those with abnormal neurological findings. A significant association between PIQ only and neurological status, and impairment of motor function was seen. For both IQ scales, there was a difference between the group with and the group without epilepsy. Statistical testing showed evidence for a significant association with PIQ and weak evidence only for an association with VIQ. However, once neurological status and motor impairment was accounted for, no significant effect of epilepsy remained. IQ scores were not associated with seizure frequency, most likely because in this sample the epilepsy was mild. Age at onset of epilepsy showed a significant association with PIQ only, and inspection of scatter plots suggested that there was a relationship between age at onset, neurological status and PIQ.

Univariate analyses provided evidence for associations between the clinical variables birth weight, prolonged oxygen supplementation and persistent arterial duct for both IQ scales. In addition, associations were identified between performance on the TOMI (i.e. motor impairment) and PIQ. Regression analyses indicated that a number of clinical variables were independent predictors of cognitive function whereas epilepsy did not independently contribute to cognitive outcome.

Part III

Structural brain abnormalities detected on magnetic resonance imaging

Part III of this thesis consists of two chapters. Chapter 7 first describes the findings identified on visual assessments of the MR images that were acquired in the context of this study. Second, the relationships of the MRI findings with the expected pathology of perinatal brain injury in preterm children and with neonatal cranial ultrasound findings in the study population are discussed. Chapter 8 first gives some theoretical background on VBM and describes the VBM methodology used in this study. This is followed by presentation and discussion of the results of the VBM analyses performed on grey matter segments for identification of subtle grey matter abnormalities, and discussion of associations of the VBM results with structural brain abnormalities identified on visual assessment of the images.

In both chapter 7 and chapter 8 the data are presented mainly in a descriptive way. The focus in this part of the thesis is on identification of brain abnormalities using visual assessment of MRI images and VBM for detection of subtle grey matter abnormalities. The associations between structural brain abnormalities identified in the population of this study and outcome (i.e. epilepsy and cognitive function) are presented and discussed in Part IV of this thesis.

Chapter 7: Detection of structural brain abnormalities by visual assessment of MR images

Magnetic resonance imaging data of 54 children (24 with epilepsy and 30 without epilepsy) were available for visual inspection. Median age at MR scanning was 130 months (min 84, max 181) and there were 31 boys and 23 girls. None of the children with epilepsy had status epilepticus within 48 hours of MR scanning. All MR images were of good quality. Fifty one datasets were acquired in the context of this study at Great Ormond Street Hospital. Three MR datasets (JC, AP, TS) were acquired at local hospitals and copies of the films were obtained for scoring in the context of this study.

7.1 Findings on visual assessment of MR images in the whole study group and for each individual subject

In this section, first the findings for the whole group are presented and for each scoring category a typical example is shown. Second, the detailed findings on visual assessment of MR images for each individual are shown in a table.

7.1.1 Findings on visual assessment of MR images for the whole group

For visual inspection, axial and coronal T2 weighted images and a T1 weighted 3D MPRAGE dataset were acquired (for details see chapter 4, section 4.4.1). The images were reviewed according to the scoring system outlined in chapter 4, section 4.4.2 (a copy of the scoring sheet can be found in appendix 2), which pays particular attention to periventricular white matter, and cortical and subcortical grey matter structures. The images were reviewed by two independent observers (Dr WK Chong, Neuroradiologist, who was unaware of the subjects' clinical details, and by the author).

Table 7.1 below summarises the findings from visual assessment for the whole group. MRI was judged as normal in 19 of the 54 children (35%). Structural brain abnormalities were identified in 35 children (65%).

Abnormalities in the periventricular white matter were seen in 32 (59%) of the 54 children. Five of the 32 children (16%) had periventricular gliosis only without white matter reduction (radiological signs of gliosis were defined as high signal on T2 weighted images; see chapter 4, section 4.4.2). Periventricular white matter reduction with or without gliosis was seen in 27 (84%) of the 32 children. In 9 (33%) of these 27 children the degree of white matter reduction was categorised as mild/moderate and in 18 (67%) as severe (i.e. involving subcortical white matter). Thinning of the corpus callosum was identified in 27 (50%) of the 54 children.

Grey matter abnormalities were identified in 20 (37%) of the 54 children. Nine of these 20 children had cortical abnormalities. In 5/20 children subcortical abnormalities (i.e. lesions of basal ganglia and/or thalamus) were seen and 14/20 had either unilateral or bilateral hippocampal abnormalities. A small cerebellum (either unilaterally or bilaterally) was seen in 7 (13%) of the 54 children.

A combination of periventricular white matter abnormalities and grey matter abnormalities was seen in 17 (30%) of the 54 children.

Table 7.1: Summary of findings identified by visual assessment of MR images in the whole group

MRI findings* **(n=54 datasets)**	**Study population** (% within study population)
Normal MRI **Abnormalities on visual assessment**	**19 (35%)** **35 (65%)**
Periventricular white matter abnormalities - **Gliosis only** - **White matter reduction (with/without gliosis)** mild/moderate (% within category white matter reduction) severe (% within category white matter reduction)	**32 (59%)** **5 (16%)** **27 (84%)** 9 (33%) 18 (67%)
Grey matter abnormalities** - **Cortical** (% within category grey matter abnormality) - **Basal ganglia/thalamus** (% within category grey matter abnormality) - **Hippocampal** (% within category greymatter abnormality) unilateral bilateral	**20 (37%)** **9 (45%)** **5 (25%)** **14 (56%)** 4/14 10/14
Combination of grey and white matter abnormalities	**17 (30%)**
Small cerebellum (uni- or bilateral small cerebellum)	**7 (13%)**
Thinning of corpus callosum	**27 (50%)**

* Categories are not mutually exclusive

** Hippocampal, basal ganglia and thalamus abnormalities=small, or abnormally high signal on T2 weighted images

The figures below show typical examples of periventricular gliosis without white matter reduction (figure 7.1), different degrees of periventricular white matter reduction (figures 7.2 a, 7.2 b; figures 7.3 a, 7.3 b), an example of a middle cerebral artery infarct (figures 7.4 a, 7.4 b), and of a malformation (schizencephaly; figure 7.5). Note that the images are displayed in neurological convention, i.e. left=left, right=right.

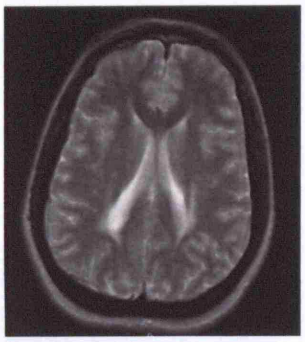
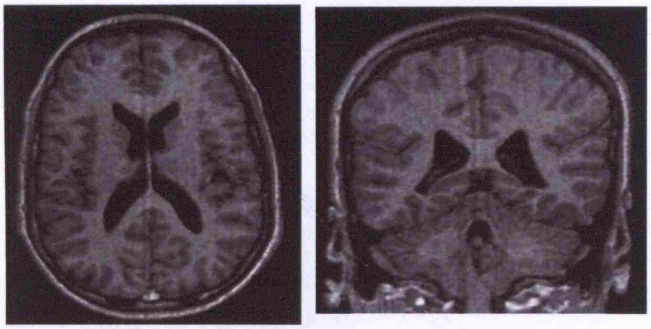
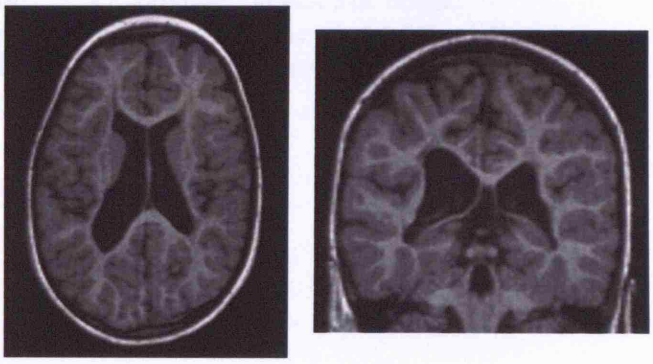


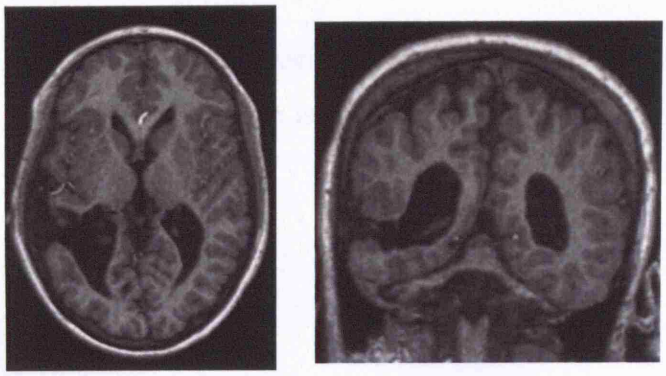
Figure 7.1: T2 weighted axial MR image.
Subject NSi; bilateral periventricular gliosis,
parieto-occipital



Figures 7.2 a and b: T1 weighted axial and coronal MR images.
Subject DHay; bilateral mild/moderate periventricular white matter reduction
parieto-occipital



Figures 7.3 a and b: T1 weighted axial and coronal MR images.
Subject EF; bilateral severe periventricular white matter reduction (l>r)
parieto-occipital and centrum semiovale, thin corpus callosum.



Figures 7.4 a and b: T1 weighted axial and coronal MR images.
Subject ML; middle cerebral artery infarct left, severe bilateral (l>r) periventricular white matter reduction, small cerebellum and pons. The small hippocampi, bilaterally small, and the thin corpus callosum are not visible on these slices.

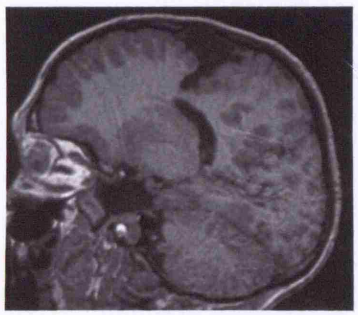


Figure 7.5: T1 weighted sagittal MR image.
Subject JW. Bilateral schizencephaly, adjacent polymicrogyria, small body of the corpus callosum.The hippocampi, bilaterally small, are not visible on this slice.

7.1.2 Findings on visual assessment of MR images for each individual child

Table 7.2 below shows for each subject the findings on visual assessment of MR images in detail. Reference to this table is made in subsequent sections.

Table 7.2: Findings on visual assessment of MR images for each child

ID	Epilepsy	MRI summary	WM	GM	Gliosis	WM reduction	Location of WM abnormality	CC	Cortex	Hippocampi	Cerebellum	Basal Ganglia Thalamus	Misc
TR	X	normal	-	-	-	-		-	-	-	-	-	
TM	X	normal	-	-	-	-		-	-	-	-	-	
TO	X	normal	-	-	-	-		-	-	-	-	-	
AP	X	normal	-	-	-	-		-	-	-	-	-	
SSk	X	normal	-	-	-	-		-	-	-	-	-	
BK	X; NNS	BG		X	-	-		small body	-	-	-	right caudate	
LB	X	WM HC	X	X	-	mild/mod bil	parieto-occipital	small body	-	bil small	-	-	
TC	X	WM	X	-	-	mild/mod bil	centrum semiovale	-	-	-	-	-	
PD	X	WM cerebellum	X		X	mild/mod bil (left>right)	centrum semiovale	small body	-	-	bil small	-	
WS	X; NNS	WM	X	-	X	mild/mod bil (left>right)	parieto-occipital centrum semiovale	small body		-	-	-	
SD	X	WM HC	X	X	-	mild/mod left	parieto-occipital	small	-	bil small	-	-	
HJ	X; NNS	WM cortex cerebellum	X	X	-	left severe	parieto-occipital centrum semiovale, frontal		MCA left	-	right small	-	
LR	X	WM	X	-	-	severe bil (right>left)	parieto-occipital	small splenium, body	-	-	-	-	
PP	X	WM HC	X	X	-	severe bil (left>right)	parieto-occipital, centrum semiovale	gen small	-	bil small	-	-	

JW	X; NNS	WM HC schizencephaly, polymicrogyria	X	X	-	severe bil	parieto- occipital	small body	schizence phaly bilateral. polymicro gyria adjacent to schizence ph.	bil small	-	--	
AM	X; NNS	WM cortex	X	X	X	severe right	parieto- occipital, centrum semiovale	gen small	right parietal	-	-	-	shunt right parietal
SH	X	WM	X	-	X	severe bil (left>right)	parieto- occipital	small body, splenium	-	-	-	--	
KS	X	WM HC cortex BG cerebellum	X	X	X	severe bil (right>left)	parieto- occipital, frontal	? agenesis	ulegyria temporal right	right small	left small	putamen, thalamus bilateral	
ML	X; NNS	WM HC cortex cerebellum	X	X	X	severe bil (left>right)	parieto- occipital, centrum semiovale, frontal	gen small	MCA left	left small	pons, cereb small (?PCH)	-	
AU	X	WM HC	X	X	-	severe bil	parieto- occipital, centrum semiovale, frontal	gen small	-	right high signal	-	-	
SHay	X	WM HC thalamus	X	X	-	severe bil (right>left)	parieto- occipital, centrum semiovale, frontal	small body, splenium	right parietal	bil small	-	thalamus left	shunt right temporo parietal
EG	X; NNS	WM HC cerebellum	X	X	-	severe bil (left>right)	parieto- occipital, centrum semiovale, frontal	gen small	-	left small	right small	-	

TS	X; NNS	WM cerebellum	X	-	X	severe bil (left>right)	frontal	gen small	-	-	right small	-	
EF	X	WM	X	-	X	severe bil (left>right)	parieto-occipital, centrum semiovale, frontal	gen small	-	-	-	-	
RK	-	normal	-	-	-	-		-	-	-	-	-	
DS	-	normal	-	-	-	-		-	-	-	-	-	
JoCol	-	normal	-	-	-	-		-	-	-	-	-	
AT	-	HC	-	X	-	-		-	-	bil small	-	-	
AC	-	normal	-	-	-	-		-	-	-	-	-	
CS	-	normal	-	-	-	-		-	-	-	-	-	
VH	-	normal	-	-	-	-		-	-	-	-	-	
CR (I)	-	normal	-	-	-	-		-	-	-	-	-	
MD	-	normal	-	-	-	-		-	-	-	-	-	
JSk	-	normal	-	-	-	-		-	-	-	-	-	
ASa	-	normal	-	-	-	-		-	-	-	-	-	
SDaW	-	normal	-	-	-	-		-	-	-	-	-	
BA	-	normal	-	-	-	-		-	-	-	-	-	
SF	-	normal	-	-	-	-		-	-	-	-	-	
JRu	-	normal	-	-	-	-		-	-	-	-	-	
BBe	-	HC	-	X	-	-		-	-	bil small	-	-	
DSk	-	WM Chiari I	X	-	-	mild bil	centrum semiovale	-	-	-	? Chiari I	-	
LWa	-	WM	X	-	X (bil)	-	parieto-occipital	small body	-	-	-	-	
NK	-	WM HC cortex	X	X	X (left)	-	parieto-occipital	small body	right temporal/ parietal	bil small	-	-	shunt right temporo parietal
LiWi	-	WM	X	-	X (bil)	-	parieto-occipital, frontal (r>l)	-	-	-	-	-	
NSi	-	WM	X	-	X (bil)	-	parieto-occipital, frontal	small body	-	-	-	-	
GO	-	WM BG,Thal.	X	X	X (right >left)	-	centrum semiovale	-	-	-	-	right caudate, left thalamus	

DHay	-	WM	X	-	-	mild/mod bilateral	parieto-occipital	-	-	-	-	-	
CT	-	WM HC	X	X	-	right severe, left moderate	parieto-occipital, centrum semiovale	gen small	-	bil small	-	-	
JC	-	WM cortex	X	X	-	right moderate, left severe	parieto-occipital, centrum semiovale, frontal	gen small	thin left parietal	-	-	-	shunt left parietal
MC	-	WM	X	-	-	mild/moderate bilateral	parieto-occipital	gen small	-	-	-	-	
LH	-	WM	X	-	X	mild/moderate bilateral (l>r)	centrum semiovale	small body	-	-	-	-	
RR (II)	-	WM BG,Thal.	X	X	-	severe left	centrum semiovale	small body	-	-	-	caudate, putamen, thalamus left	
LO (I)	-	WM HC cortex cerebellum	X	X	-	severe right	parieto-occipital, centrum semiovale	gen small	left parietal	left small	bil small	-	shunt left parietal
CO (II)	-	WM	X	-	-	severe bil (r>l)	right:parieto-occipital, centrum semiovale, frontal, left: parieto-occipital, frontal	gen small	-	-	-	-	

WM = periventricular white matter lesion, either white matter reduction or gliosis or both; WMR = periventricular white matter reduction, classification of severity see chapter 4, section 4.4.2. CC=corpus callosum. GM = in this summary includes cortical, hippocampal abnormalities (HC, as defined in the methods chapter), basal ganglia lesions (BG), thalamus lesions (Thal). Cereb= cerebellum, misc = miscellaneous, bil = bilateral, gen = generally, mod = moderate, MCA = middle cerebral artery infarct, PCH = ponto-cerebellar hypoplasia. NNS = neonatal seizures

7.2 Location, extent and type of lesions

In this section, the findings within the three main categories "periventricular white matter abnormalities", "grey matter abnormalities", and "combination of white and grey matter abnormalities" are presented in more detail with regard to location, extent and type of lesions.

7.2.1 Periventricular white matter abnormalities

7.2.1.1 Gliosis without white matter reduction

In three of the five children with gliosis only on visual assessment, the gliotic changes were located in the parieto-occipital white matter (LWa, NK) and in one child in the centrum semiovale (GO). In two children gliosis was more extensive, involving frontal and parieto-occipital white matter (NSi, LiWi). In all but one child (NK, left sided gliosis) the gliotic changes were seen bilaterally (asymmetrical in one child, GO). Three of the children (LWa, NK, NSi) with periventricular gliosis also had a small body of the corpus callosum on visual inspection of MRI images. In one child (GO) additional signal abnormalities on the T2 weighted images in the right caudate and the left thalamus were seen, and another child (NK) had bilaterally small hippocampi and a focal cortical lesion (caused by a shunt in the left temporal parietal region).

7.2.1.2 White matter reduction with or without gliosis

The degree of white matter reduction was judged as mild or moderate in 9 (33%) children (LB, TC, PD, WS, SD, DSk, DHay, MC, LH) and as severe in 18 (67%; for details see table 7.2) of the 27 children. In 9 (33%; PD, WS, AM, SH, KS, ML, TS, EF, LH) of the 27 children with periventricular white matter reduction, associated gliotic changes were seen.

In 7 (26%; LB, SD, LR, JW, SH, DHay, MC) of the 27 children white matter reduction was confined to the parieto-occipital area (4 with mild/moderate, 3 with severe reduction of white matter). In one (SD) of these seven children, white matter reduction was only seen on one side and in the other six children bilaterally (in 2/6 children, LR, SH, the distribution was asymmetrical). In one child (TS), only frontal white matter was affected (severe reduction, bilateral asymmetrical distribution). In 5 (19%; TC, PD, DSk, LH, RR) of the 27 children, only white matter in the region of the centrum semiovale (region adjacent to the body of the lateral ventricle) was affected (4 with mild/moderate, 1 with severe white matter reduction). In one of these five children (RR), white matter reduction was only seen on one side and in four children (TC, PD, LH, DSk) it was bilateral (asymmetrical in two children; PD, LH).

More widespread white matter reduction (i.e. more than one region affected) was seen in 14 (52%; see table 7.2 for details) of the 27 children. In five children (WS, PP, AM, CT, LO), the parieto-occipital regions and centrum semiovale were involved (mild/moderate in one, severe in four children). In three (WS, PP, CT) of the five cases, the distribution was bilateral (all asymmetrical) and in the remaining two cases there was only unilateral reduction of white matter. One child (KS) had asymmetrical severe white matter reduction in the parieto-occipital and frontal periventricular white matter. In 8 (HJ, ML, AU, SHay, EG, EF, JC, CO) of the 14 children, parieto-occipital white matter as well as white matter in the centrum semiovale and frontal periventricular white matter was affected. The degree of white matter reduction in all eight children was judged as severe. In seven of these children both sides were affected (in 6/7 asymmetrical), and in one child only unilateral involvement was seen.

In three (11%, TC, DSk, DHay) of the 27 children, periventricular white matter reduction was the only abnormality detected on visual inspection of MR images (in one of these three children, DSk, however, there was also suspicion of a mild Chiari I malformation). In all three children, the degree of white matter reduction was judged as mild/moderate. In 7 (26%) of the 27 children with periventricular white matter reduction (3/7 with

mild/moderate, 4/7 with severe white matter reduction), partial or general thinning of the corpus callosum was seen as the only additional abnormality.

In 15 (56%) of the 27 children, grey matter abnormalities were seen in combination with periventricular white matter reduction. In 10 (LB, SD, HJ, PP, AM, AU, EG, CT, RR, JC) of the 15 children, one grey matter structure was affected (hippocampal abnormality, either unilaterally or bilaterally, n=6; basal ganglia or thalamus n=2; focal cortical lesion n=3). In 5 (JW, KS, ML, SHay, LO) of these 15 children, more than 1 grey matter structure was affected (see table 7.2 for details). In two (NK, GO) of the five children with periventricular gliosis only, grey matter lesions were seen too (see section 7.2.1.1). The grey matter findings are discussed in more detail below.

Seven children had a unilaterally or bilaterally small cerebellum associated with periventricular white matter reduction. In only one child (PD), white matter reduction was mild/moderate, in all other children it was judged as severe. In five (ML, KS, LO, HJ, EG) of the six children with a small cerebellum and severe white matter reduction, cortical lesions, hippocampal, or basal ganglia/thalamus lesions were also seen.

7.2.2 Grey matter abnormalities

7.2.2.1 Isolated grey matter abnormalities

In 2 (AT, BBe) of the 54 children, abnormalities in only one grey matter structure and no combination with periventricular white matter abnormalities were seen. In both cases the hippocampi were judged as small bilaterally. In one child (BK), an isolated lesion in the right caudate (high signal on T2 weighted images) was seen. This child also had a small body of the corpus callosum but no periventricular white matter abnormalities.

7.2.2.2 Grey matter abnormalities in combination with periventricular white matter lesions

As described above in section 7.2.1.2, in 17 children grey matter abnormalities were seen in combination with periventricular white matter abnormalities. In this section these grey matter abnormalities are discussed in more detail.

7.2.2.2.1 Cortical grey matter

Focal cortical grey matter abnormalities (either isolated or in combination with basal ganglia, thalamus and/or hippocampal abnormalities) were seen in 5 of the 17 children. In these five children, the cortical lesion was at the site of an in situ ventriculo-peritoneal shunt (AM, SHay, NK, JC, LO). In two (AM, JC) of these five children, this focal cortical lesion was the only grey matter abnormality, and in three children (SHay, NK, LO) at least one other grey matter abnormality was seen. In all children with a shunt but one (NK; gliosis without white matter reduction), the degree of periventricular white matter reduction was judged as severe.

More widespread cortical grey matter abnormalities were seen in four children. In two children (HJ, ML) a middle cerebral artery (MCA) infarct was seen on MRI. One child (HJ) with an MCA infarct had a small cerebellum on the contra lateral side, and in the other child (ML) pons and cerebellum were judged as small and a possible diagnosis of ponto-cerebellar hypoplasia was raised. In both children, the degree of periventricular white matter reduction was judged as severe. A neuronal migration disorder (bilateral schizencephaly with adjacent polymicrogyria, see figure 7.5) was identified in one child (JW). The degree of periventricular white matter reduction in this case was judged as severe. In one child (KS), a complex combination of lesions was identified, consisting of unilateral ulegyria, focal cortical abnormality at the site of a ventriculo-peritoneal shunt on the contra lateral side, bilateral signal abnormalities in putamen and thalamus, unilaterally small cerebellum on the contra lateral side to the ulegyria, unilaterally small hippocampus,

severe periventricular white matter reduction and a possible agenesis of the corpus callosum.

7.2.2.2.2 Hippocampi

Hippocampal abnormalities (either small hippocampi or abnormal signal on T2 weighted images) were seen in 14 (26%) of the 54 children. Four of the 14 children had unilateral and 10 had bilateral hippocampal abnormalities. In only two children (AT, BBe), the hippocampal abnormalities were the only abnormalities identified on visual analysis of MRI. In 12 children hippocampal abnormalities were combined with white matter and/or other grey matter abnormalities. In 13 of the 14 children, the hippocampi were judged as small and only in 1 child (AU) abnormally high signal on T2 weighted images was seen.

7.2.2.2.3 Basal ganglia and thalami

Abnormalities in the basal ganglia and/or thalami (either small basal ganglia/thalami or abnormal signal on T2 weighted images in basal ganglia/thalami) were identified in five children (BK, KS, SHay, GO, RR). In three children, these abnormalities were present on one side only.

In one child (BK), the abnormalities in the basal ganglia (abnormal signal in the right caudate) were seen without other associated grey matter abnormalities or periventricular white matter abnormality. In two children (KS, SHay), severe white matter reduction and cortical abnormalities were present. In the other two children (GO, RR) periventricular white matter abnormalities but no other grey matter abnormalities were seen.

The thalami (either unilaterally or bilaterally) were affected in four of the five children, and in all cases except one (SHay) in combination with basal ganglia abnormalities.

7.2.3 Cerebellum

The main focus of the visual assessment of the MR images was on identification of periventricular white matter abnormalities and supratentorial grey matter abnormalities. However, if there were abnormal findings infratentorially these were noted, too.

In seven children, the cerebellum was judged as small either on one side (HJ, KS, EG, TS, LO) or both sides (PD, ML). In all children this was combined with severe periventricular white matter reduction, except in one case (PD), in which there was mild/moderate white matter reduction.

In one child (WS, included in the seven cases above), in addition to a small cerebellum, the pons was slightly small and the findings were interpreted as possible ponto-cerebellar hypoplasia. However, this child also had an MCA infarct and severe periventricular white matter reduction. In one child (DSk), the possible diagnosis of a mild Chiari I malformation was raised.

In four of the seven cases, the small cerebellum was seen on the contra lateral side to the most severely affected side of periventricular white matter lesions or the MCA infarct (HJ) respectively.

7.2.4 Corpus callosum

In 27 (50%) of the 54 children, the corpus callosum was judged as thin (generally or parts of the corpus callosum). In only one child (BK), thinning of the corpus callosum was not associated with periventricular white matter abnormalities. In three children (NS, LWa, NK), thinning of the corpus callosum was associated with periventricular gliosis without visually obvious white matter reduction, and in 23 children with periventricular white matter reduction (6 with mild/moderate, 17 with severe white matter reduction). In one child (KS), the findings were interpreted as possible agenesis of the corpus callosum.

7.3 Primary lesions and associated pathology in perinatal brain injury

As outlined in chapter 3, the central pathology in the preterm brain is white matter damage. Histopathological studies have shown that ischaemic and/or haemorrhagic damage can also occur in other locations such as the cortex, the basal ganglia and thalami, brainstem, hippocampus, and cerebellum. Frequently, these lesions occur in combination and in association with white matter damage. The aim of this section is to examine in more detail whether, and if so, with what frequency, in the study population such associated pathologies are identified on visual assessment of MR images. In addition, MR findings in the study population that are not primarily expected in the context of perinatal brain injury are described.

7.3.1 Associated lesions in preterm perinatal brain injury

The typical primary perinatal brain injury pattern in preterm children consists of periventricular white matter damage, i.e. gliosis, reduction of white matter with or without gliosis and often associated thinning of the corpus callosum. This typical pattern was seen in 32 (91%) of the 35 children in whom abnormalities were identified on visual assessment of MR images. As described in detail above, in 17 (53%) children this was combined with lesions in one or more than one other location (i.e. abnormalities in basal ganglia, thalami, hippocampi, cerebellum, cortex). Four of these 17 children had, in addition to the white matter abnormalities, other pathologies that are not typically expected in a preterm population (see section 7.3.2 below).

In 10 children, lesions that are compatible with the pattern described as associated injury in perinatal preterm brain injury were seen in one location (basal ganglia/thalamus in two children, GO, RR; hippocampi in six, LB, SD, PP, AU, CT, NK; cerebellum in two children, PD, TS). In three children, associated lesions in more than one location were seen (basal ganglia and hippocampi in one child, SHay; cerebellum and hippocampi in two

children, EG, LO). In three children (BK, AT, BBe), lesions of basal ganglia or the hippocampi were seen without associated periventricular white matter abnormality.

Focal cortical lesions were seen in five children. These were associated with a shunt inserted for treatment of post-haemorrhagic hydrocephalus (see table 7.2 and section 7.2.2.2.1). In all cases but one (NK), this was seen in the context of severe reduction of periventricular white matter and thus not considered as an associated pathology but rather as a direct consequence of haemorrhagic white matter injury.

7.3.2 Lesions identified on MRI that are not typically seen in the context of preterm perinatal brain injury

In 4 (11%; JW, ML, HJ, KS) of the 35 children, the main lesion seen on MRI was not of the type that is typically seen in the context of perinatal brain injury in preterm children. Schizencephaly is a structural brain abnormality, characterised by clefts spanning the cerebral hemispheres from the pial surface to the lateral ventricle and lined by heterotopic grey matter (in this case polymycrogyria). Agenesis of the corpus callosum, suspected in one child (KS), can also be timed at very early gestational age. However, in this child this was combined with ulegyria and abnormalities of thalami and putamen, a lesion pattern which is seen after a hypoxic-ischaemic encephalopathy at term age. Furthermore, in this child periventricular white matter abnormalities were seen (severe bilateral white matter reduction), i.e. the injury pattern expected in a preterm child with perinatal brain injury. This child, too, had an additional cortical lesion associated with a shunt. It is likely that in this case more than one insult occurred at different time points. In two children (HJ, ML), a middle cerebral artery (MCA) infarct was identified as the main lesion. The severity of injury caused by MCA infarcts depends on which parts of the MCA are affected, i.e. when the distribution of the stem of the MCA is affected, cortex, white matter, basal ganglia and internal capsule (posterior limb) are affected, whereas when only a cortical branch of the MCA is affected, the basal ganglia, white matter and internal capsule might be spared. In both cases the injury following the infarct did not involve the basal ganglia. The

periventricular white matter abnormalities seen in both children might be part of the injury caused by the MCA infarct or caused by a separate perinatal insult typically seen in preterm children. In one of these two children (ML) the MRI findings, in addition to the MCA infarct, were suggestive of ponto-cerebellar hypoplasia. However, laboratory investigations did not show abnormal results or indicate a metabolic or genetic cause (however, it has to be kept in mind that these investigations are often negative in ponto-cerebellar hypoplasia). Thus it seems more likely that cerebellum and pons remained small as a secondary lesion.

7.4 MRI findings and neonatal cerebral ultrasound findings

In this section, MR findings are presented in relation to the neonatal cranial ultrasound diagnoses. This is focused on periventricular white matter lesions based on the ultrasound classification system introduced in chapter 4, and therefore structures such as the basal ganglia, thalami, the hippocampi, corpus callosum, or cerebellum are not included in this comparison. The details of the neonatal ultrasound findings for each study participant are given in appendix 4.

Table 7.3: Neonatal cranial ultrasound diagnoses and MRI categories based on visual assessment of images (at age 9-13 years)

		MRI normal n=19	MRI abnormal n=35
Normal ultrasound	**n= 7**	**7**	**-**
Non-parenchymal lesions*	**n=21**	**9**	**12**
- GLH	n= 7	3	4
- IVH II-III	n= 7	2	5
- Combination of GLH, IVH II-III	n= 3	1	2
- mild/moderate transient VD	n= 4	3	1
Parenchymal lesions	**n=26**	**3**	**23**
- Cystic PVL	n= 6	2	4
- HPI	n= 4	1	3
- Severe persistent VD	n= 1	-	1
- Combination with non-parenchymal lesion on contralateral side**	n=15	-	15

VD= ventricular dilatation, GLH=germinal matrix haemorrhage, IVH=intraventricular haemorrhage, HPI= haemorrhagic parenchymal infarction. cPVL= cystic periventricular leukomalacia.

* Not associated with parenchymal lesions

** Any type of parenchymal lesions combined with any type of non-parenchymal lesion on contra lateral side.

Seven children (TR, TM, TO, DS, SDaW, JSk, SF) had normal neonatal ultrasound and in all seven children visual assessment of MR images did not reveal any brain abnormalities.

Forty seven children had abnormal ultrasound findings (for details see appendix 4). In 12 (26%) of those, MRI was normal and in 35 (74%) visual assessment of MR images revealed brain abnormalities. In the majority (9/12) of those in whom neonatal ultrasound was abnormal and MRI at the time of this study was visually assessed as normal, the ultrasound lesions were non-parenchymal. In 3 of the 12 children ultrasound findings had been interpreted as parenchymal lesions (JoCol: cystic PVL bilaterally, CS: cystic PVL bilaterally, JRu: unilateral HPI).

In the following sections, the abnormalities identified on MRI in relation to neonatal ultrasound findings are described in more detail.

7.4.1 Non-parenchymal lesions

Non-parenchymal lesions (defined as GLH, IVH II, IVH III or isolated transient mild/moderate ventricular dilatation) on neonatal ultrasound were diagnosed in 21 children. In 9 (43%) of these 21 children MRI was normal on visual assessment and in 12 (57%) it was abnormal.

In four children (AP, LB, MD, ASa) isolated mild or moderate transient ventricular dilatation was seen as the only abnormality on neonatal ultrasound. In three of these four children (AP, MD, ASa), MRI was normal on visual assessment. In one of these four children (LB) mild bilateral periventricular white matter reduction, a partially small corpus callosum and bilaterally small hippocampi were seen on MRI.

In 17 (for details see appendix 4) of the 21 children, GLH, IVH II, IVH III, or a combination of these non-parenchymal lesions was diagnosed by neonatal cranial ultrasound. Six of these 17 children (SSk, RK, AC, VH, CR, BA) had normal MRI findings. In 2 of the 17 children (AT, BBe), the only abnormality on MRI were bilaterally small hippocampi. In four children (TC, WS, SD, DSk), mild/moderate periventricular white matter reduction was identified on MRI. In one of these four children (DSk), findings on MRI suggested a mild Chiari I malformation.

Three of the 12 children with abnormal MRI following non-parenchymal lesions on neonatal ultrasound had signs of periventricular gliosis on MRI. In one of these three children (NK), in addition to a non-parenchymal lesion, moderate and transient ventricular dilatation had been noted on neonatal ultrasound, and on the MRI a focal cortical lesion at the site of a shunt was noted in addition to the periventricular gliosis. In two of the 12

children with abnormal MRI (SH, AU), severe periventricular white matter reduction was seen on MRI.

Overall, within the ultrasound category non-parenchymal lesions, as given by table 7.3, there was no clear pattern identifiable regarding the individual lesions (i.e. grade of haemorrhage or combination of individual lesions) and specific corresponding MRI findings. It has to be kept in mind though that the numbers in the individual categories were very small.

7.4.2 Parenchymal lesions

Twenty six (for individual cases see appendix 4) children had parenchymal lesions (defined as cystic PVL, HPI, severe persistent ventricular dilatation) with or without non-parenchymal lesions on the contra lateral side on neonatal ultrasound. MRI was normal on visual assessment in 3 (12%) children (JRu, JoCol, CS) and abnormal in 23 (88%) children (see table 7.3 above).

In one (BK) of the 23 children with abnormal MRI, only a focal lesion in the right caudate and a partially small corpus callosum was seen on MRI. In 22 children periventricular white matter abnormalities were detected on MRI. In 2 (NSi, GO) of the 22 children, isolated periventricular gliosis was seen on MRI and in 20 children periventricular white matter reduction was detected. The degree of white matter reduction was mild or moderate in 4/22 (PD, DHay, MC, LH) and severe in the remaining 16 children. Similarly to the non-parenchymal lesions, there was no clear pattern identifiable regarding the individual ultrasound lesions (i.e. cystic PVL, HPI, or combination of lesions) and specific corresponding MRI findings.

Within the group of children with parenchymal lesions on neonatal ultrasound, MRI revealed bilateral schizencephaly with adjacent polymicrogyria, in one child (JW). Neonatal ultrasound findings in this child had been interpreted as bilateral cystic PVL and

no cortical abnormality was noted. In two children (ML, HJ), a left sided middle cerebral artery infarct was seen on MRI and in both children this had not been detected on serial neonatal ultrasound examinations (in both children a parenchymal lesion was seen on ultrasound, in one case left sided HPI and in the other case left sided cPVL with IVH III bilaterally). In these two children, MRI showed, in addition to the MCA infarct, severe left sided (HJ) or bilateral (ML) white matter reduction.

7.4.3 Bright echoes

Bright echoes on neonatal ultrasound were not taken into account in the descriptive analyses described above since in no case did bright echoes appear in isolation but always in the context of non-parenchymal or parenchymal lesions as defined in chapter 4.

7.5 Distribution of visible brain abnormalities on MR images between the group with and the group without epilepsy and in relation to cognitive function

This is described and discussed in detail in chapter 9 and chapter 10.

7.6 Summary

In this selected group of preterm children, 35% had normal and 65% had abnormal findings on visual assessment of MR images. In 59% of the 54 children (91% of the 35 children with abnormal MRI) periventricular white matter abnormalities, i.e. the typical primary lesion pattern of perinatal brain injury in preterm children, were identified. This was combined with partial or general thinning of the corpus callosum in the majority of the children and most of those children had severe reduction of periventricular white matter.

Associated pathologies such as basal ganglia, thalamus, hippocampal, and/or cerebellar abnormalities that have been shown in histopathological studies to occur in preterm children in the context of perinatal brain injury were identified on visual assessment of MRI in a large propotion of the children with abnormal MRI. However, the cortical lesions (focal and more widespread) seen in this study population were of a different nature from those described as associated pathologies in preterm perinatal brain injury in the literature.

In 11% of the 35 children with abnormal MRI, the main lesion on MRI was not of the injury pattern that is typically expected in preterm children but consisted of lesions including arterial infarcts, disorders of neuronal migration and cortical organisation, and lesion patterns that indicated injury at different time points.

In all children with normal neonatal ultrasound, visual assessment of MRI did not identify any structural brain abnormalities. In 43% of those with non-parenchymal lesions on neonatal ultrasound, MRI was normal, and in 57% at least one abnormality was seen on MRI. In 12% of those with parenchymal ultrasound lesions MRI was normal and in 88% abnormalities were seen on MRI.

7.7 Discussion

The main aim of this chapter was first to describe the findings identified by visual inspection of MR images that were obtained at the time of this study (age 9-13 years) in the whole study population (54 children). The identified brain abnormalities were grouped and it was attempted to identify patterns of lesions. Second, relationships between identified lesion patterns detected in the study population and the expected brain pathology in a preterm population were presented, and relationships with the neonatal cranial ultrasound findings were described.

It has to be kept in mind that the group of preterm children included in this study is selected and not a random group. All preterm children in this study were treated at one tertiary

center and selected on the basis of the presence (the group with epilepsy) or absence (the control group) of an impairment (epilepsy). The children without epilepsy were selected so that their neonatal ultrasound findings matched the ultrasound findings of those with epilepsy as closely as possible. Therefore a direct comparison with the results from existing studies that investigate unselected preterm groups, either in hospital or population based studies, is likely to be limited.

7.7.1 Findings on visual assessment of MR images in the whole group

Structural brain abnormalities were identified in a high percentage (65%) of the children included in this study. This high proportion of abnormal findings on visual assessment of MR images is most likely due to the fact that the study population is not a random but selected sample. Nevertheless, the numbers reported in previous studies are not too dissimilar to those in the current study. For example, Stewart et al (1999), in a non-selected cohort of 109 preterm children born before 33 weeks of gestation, found clear abnormalities on MRI (performed at age 14-15 years) in 55 %, and equivocal findings (defined as negligible ventricular dilatation, negligible thinning of the corpus callosum, isolated white matter signal change) in a further 21% of the children. Skranes et al (2005) reported a very high percentage of structural abnormalities (in 84% of an unselected group of 55 preterm children born at 25-32 weeks of gestation and imaged at the age of 15 years), mainly in the periventricular white matter. The percentage of abnormal findings on MR scans at age 15 years in a group of 87 preterms in the study by Cooke and Abernethy (1999) was somewhat lower, at 43%. The differences in the findings are most likely due to the fact that children with severe motor impairment were excluded from the study by Cooke and Abernethy (1999). In addition, it is likely that different criteria for classification of MR findings in individual studies may caused a relatively large variation in findings reported.

Inder et al (2003) reported a high incidence of brain abnormalities, mainly in the white matter, at term equivalent age in an unselected regional population of infants born between 23-32 weeks of gestation. Approximately two thirds of the infants had abnormalities on

visual inspection of MR images. Similarly, in another recent study investigating a large unselected cohort of preterm infants born at less than 30 weeks of gestation with serial MRI brain scans from birth to term age (age at term equivalent scanning ranging from 36-53 weeks postmenstrual age), Dyet et al (2006) reported a high incidence of abnormalities. Out of 87 infants who had MRI at term equivalent age, only 8% had normal MRI findings. In their cohort, diffuse white matter injury was the most common finding (seen in 80% of the term age MR images), followed by ventricular dilatation, punctate white matter lesions, basal ganglia and thalamic lesions. Focal lesions in the periventricular white matter such as haemorrhagic parenchymal infarction and periventricular leukomalacia were not very frequently seen on the term age scans. It has to be kept in mind though, that these studies, in contrast to the current study and the studies by Stewart et al (1999), Skranes et al (2005), and Abernethy and Cooke (1999), focused on very early MRI, i.e. at term equivalent age. It is possible that between term age and childhood/adolescence lesion characteristics change (for example, development of gliosis following an ischaemic insult to the periventricular white matter). In addition, developmental changes in the brain may occur, which might also cause changes in lesion characteristics over time (see e.g. Skranes et al, 1998). Furthermore, the infants investigated in the studies by Inder et al (2003) and Dyet et al (2006) were born almost two decades later than the children investigated in the above mentioned study by Stewart et al (1999), and almost a decade later than the children included in the current study. This makes comparison between studies performed at different time points additionally difficult.

The majority of abnormalities seen in the population of the current study were periventricular white matter lesions with only a small proportion having gliosis only (high signal on T2 weighted images in the periventricular white matter without reduction of white matter) and the majority having reduction of periventricular white matter of different degree. This was frequently seen in association with thinning of the corpus callosum. These findings, i.e. that the majority of the abnormalities are located in the periventricular white matter, are consistent with those of previous studies performed in childhood or adolescence (e.g. Stewart et al, 1999; Krägeloh-Mann et al,1999; Cooke and Abernethy, 1999; Skranes et al, 1998, 2005).

The majority of grey matter abnormalities in the population of the current study were seen in association with white matter lesions and not in isolation. This, too, is consistent with results of previous studies on MRI findings in preterm infants and children (e.g. Inder et al, 2003; Skranes et al, 1998, 2005) and with findings obtained from histopathological studies (e.g. Friede, 1989; Paneth et al, 1994; Marin-Padilla, 1996, 1997, 1999).

7.7.2 Location, extent, type of lesions and associated lesions

As outlined in the previous section, the main lesion pattern identified on visual inspection of MR images was injury to the periventricular white matter. The majority of the children with abnormalities in the periventricular white matter had white matter reduction with or without associated gliosis and only a minority had gliotic changes only. Consistent with findings reported in previous MRI studies (e.g. Krägeloh-Mann et al, 1999; Skranes et al, 2005) the site of lesion seen most frequently was the parieto-occipital white matter. The majority of the children with white matter reduction had a severe degree of reduction of white matter, and in a high proportion of those the lesions were more widespread, i.e. involved more that one periventricular area (frontal parieto-occipital, centrum semiovale). Reduction of white matter in the periventricular area, periventricular gliotic changes, irregular ventricular margins and ventricular dilatation on MR images of preterm infants or children can be regarded as correlates of long term neuropathological changes following inflammatory/ischaemic and/or haemorrhagic perinatal injury (see also chapter 3, section 3.2.1.3 and section 3.2.2.3). The locations of the periventricular lesions seen on the MR images in this study are consistent with the locations described in neuropathological studies (e.g. Friede, 1989) with the areas adjacent to the frontal horns, the lateral corners of the lateral ventricles and the lateral surfaces of the occipital horns being the most frequently affected sites in focal white matter injury. This widespread distribution of white matter abnormalities and the high proportion of children with severe white matter reduction in the population of the current study might be partly influenced by the selection criteria applied in this study.

Partial or general thinning of the corpus callosum was a frequent finding. In all children but one, this was seen in association with periventricular white matter reduction, and in most of these cases the degree of reduction was judged as severe. Thinning of the corpus callosum (determined either qualitatively or quantitatively), in particular, thinning of the posterior part has been described in several previous studies (Cooke and Abernethy 1999; Stewart et al, 1999; Nosarti et al, 2004). In most studies, corpus callosum abnormalities have been seen in association with ventricular dilatation and periventricular white matter reduction, and it has been suggested that thinning of the corpus callosum can be regarded as a secondary rather than primary lesion (e.g. Stewart et al, 1999). However, Nosarti et al (2004), who assessed corpus callosum size on MR images of preterm children at age 14-15 years quantitatively, found evidence for selective thinning of the corpus callosum in the posterior part even after accounting for white matter volume and ventricular size. The authors argue that this indicates that thinning of the corpus callosum can occur independently of other brain lesions in preterm children.

In seven children within the current study, the cerebellum was judged as small, either unilaterally or bilaterally. In all cases, this was associated with periventricular white matter reduction. Cerebellar atrophy, identified qualitatively or quantitatively on MRI, has been reported in previous publications on brain abnormalities in preterm children, and it has been shown that this is not an unusual finding in the context of perinatal brain injury (Mercuri et al, 1997; Krägeloh-Mann et al, 1999). Allin et al (2005) found an association between cerebellar volume and reduced white matter volume in 14-15 year old preterms. Volumetric MRI studies performed in preterm infants at term equivalent age suggest that cerebellar volume in comparison to term born controls is only decreased when supratentorial pathology, i.e. haemorrhagic parenchymal infarction, periventricular leukomalacia, or ventricular dilatation is present (e.g. Srinivasan et al, 2006). In a study performed by Shah et al (2006), in which cerebellar volumes were measured at term age in preterm and term born control infants, the findings suggested that the cerebellar abnormalities were not caused by a primary injury but were a secondary effect of white matter injury independently of immaturity. Neuropathological studies (see e.g. Paneth et al, 1994) have

reported cerebellar lesions, in particular haemorrhage, as associated lesions with periventricular white matter injury in preterm children. In addition, atrophy, identified on MR images later in life, may represent the end stage lesion after cerebellar haemorrhage in the perinatal period.

In about a third of the children grey matter abnormalities were identified on visual assessment of MR images. In the majority of the cases, these abnormalities were seen in association with periventricular white matter lesions. Cortical lesions consisted mainly of focal lesions in association with ventriculo-peritoneal shunt insertion for posthaemorrhagic ventricular dilatation (all but one of these children had signs of haemorrhage on the neonatal ultrasound). Therefore, these cortical lesions are not to be regarded as primary injury sustained in the context of perinatal brain injury but a consequence of primary white matter injury associated with therapeutic intervention. However, in the majority of the children with a shunt, abnormalities in at least one other grey matter structure was seen, possibly indicating (in addition to the severe white matter reduction) that more widespread perinatal injury was present. More extensive cortical lesions were seen in two children with infarcts, one child with a neuronal migration disorder and one child with a very complex pattern of lesions suggesting injury at different time points. The abnormalities present in these children are not typical for those seen in preterm children in the context of perinatal brain injury and will be discussed in more detail below.

As discussed in detail in chapter 3, there is evidence from neuropathological studies that injury to immature brain has effects on subsequent brain development and that both white and grey matter is affected. These brain abnormalities consist of changes to the microstructure of the brain (see chapter 3, section 3.2.3.1, 3.2.3.2, 3.2.3.3). Thus it is not surprising that they may not be identified by purely visual analysis of MR images. Results from some more recent quantitative MRI studies focusing on measurement of grey matter volume (e.g. Inder et al, 1999; Peterson et al, 2000; Allin et al, 2004), indicating decreased global or regional grey matter volumes in preterm infants, children, and even young adults, may indirectly reflect these subtle changes in grey matter structure after a perinatal insult.

In 26% of the children hippocampal abnormalities were seen, i.e. either small hippocampi or abnormally high signal on T2 weighted images. In only one of these children abnormal signal on T2 weighted images was seen; in all other cases the hippocampi were judged as small. In the majority of the children, these hippocampal abnormalities were bilateral and seen in association with abnormal findings in other brain structures, mainly periventricular white matter lesions. In histopathological studies of neonatal brains hippocampal abnormalities have been described in preterm and term infants in the context of pontosubicular necrosis (PNS, Skullerud and Westre, 1986; Friede, 1989), a lesion pattern involving the subiculum of the hippocampus and the grey matter of the pons, often associated with lesions in the neocortex, cerebellum, thalamus and white matter. These findings indicate that hippocampal abnormalities are most likely to be present in cases in which there has been a more extensive injury with subsequent widespread lesion. This is supported by results of studies using MRI imaging in preterms later in life. Peterson et al (2000), who examined preterm children at age eight years, found smaller volumes of cortical areas, hippocampi, basal ganglia, cerebellum and corpus callosum on volumetric MRI measurements when compared to term born controls. Similarly, Isaacs et al (2000) identified smaller hippocampal volumes bilaterally in a group of 11 preterm adolescents (all were neurologically normal) when compared to term born controls. In only three of these children the hippocampi had been judged as small on visual inspection of MR images. The majority of the preterm children in this study had some (mild) white matter abnormalities on visual inspection of the MR images, possibly indicating the presence of more widespread damage.

In the current study, abnormalities in basal ganglia and/or thalami were seen in five children. In all but one of these children, these lesions were seen in combination with white matter reduction. It is likely that these abnormalities, which mainly consisted of atrophy, are secondary to white matter injury and reflect the severity of the insult. The pattern seen in the current study is consistent with the findings of several previous studies. For example, Dyet et al (2006), in a cohort of preterm infants imaged serially in the neonatal period, found on visual inspection of images obtained at term equivalent age, abnormalities (mainly atrophy) in the caudate and/or thalamus that were not typical lesions of an acute

hypoxic-ischaemic or haemorrhagic injury and were seen in combination with severe white matter atrophy. Similarly, Krägeloh-Mann et al (1999), in a study investigating preterm children between the age of five and seven years, found that thalamus abnormalities were almost exclusively seen in association with severe periventricular white matter lesions. A recent quantitative MR study (Boardman et al, 2006) identified impaired growth of the thalamus and nucleus lentiformis in preterm infants (at term age) with diffuse white matter damage. The findings of the current study and the above mentioned studies are consistent with earlier findings of autopsy studies (see e.g. Paneth, 1994) that reported non-haemorrhagic thalamus and basal ganglia lesions in association with haemorrhagic white matter damage. However, it is not yet entirely clear whether these associated lesions are secondary to primary white matter damage or whether there is a common determinant in the pathogenesis of injury to locally separate brain structures.

7.7.3 Abnormalities identified on visual inspection of MR images that are unexpected in the context of perinatal brain injury in a preterm population

In four children, the lesions seen on the MR images did not reflect the typical pattern one would expect in the context of perinatal brain injury in the preterm infant/child. In one child bilateral schizencephaly with adjacent polymicrogyria was identified. In the original reports, schizencephaly is regarded as a true brain malformation and described as a structural brain abnormality characterised by clefts that span from the cerebral hemispheres from the pial surface to the lateral ventricle. The clefts are lined by heterotopic grey matter. However, the classification of schizencephaly as a true malformation is still under debate. The exact etiology of most cases is uncertain and different causative factors have been described, including ischaemic cortical injuries, viral infections, toxic and genetic factors. Schizencephaly is caused by impaired neuronal migration and cortical organisation, mechanisms that take place early in brain ontogenesis (not later than the third month of gestation), i.e. earlier than expected in the context of typical perinatal brain injury in the preterm infant.

Another child had a complex combination of lesions including abnormalities that are associated with very early gestational stages such as agenesis of the corpus callosum and also abnormalities associated with later gestational ages such as ulegyria, combined with thalamus and basal ganglia lesions (a lesion pattern seen after hypoxic-ischaemic encephalopathy at term age; Friede, 1989). This complex pattern of lesions suggests that injury occurred at more than one time point.

Infarcts of the middle cerebral artery were seen in two children. Although MCA infarcts are not typically seen in the context of preterm birth, the occurrence of this vascular injury has been reported to occur in preterm infants (deVries et al, 1997). The results of the study by deVries et al (1997) suggested that there might be an association between the distribution of the infarct and gestational age, in that in preterm infants more often involvement of other branches than the main branch is seen. In both children in the current study abnormalities in cortex, white matter and internal capsule were seen (the basal ganglia were spared), suggesting that more than just a cortical branch was affected. In one of these children pons and cerebellum were suggestive of ponto-cerebellar hypoplasia. However, laboratory investigations did not show any abnormal results with regard to an underlying metabolic or genetic cause. Thus it seems likely that cerebellum and pons remained small as a secondary lesion. All four children had periventricular white matter injury in a distribution that is typically seen in preterm brain injury. One could therefore speculate that these children, in addition to the primary pathologies described above, also suffered from hypoxic-ischaemic or haemorrhagic injury in the neonatal period.

7.7.4 Findings on MRI in childhood and neonatal cerebral ultrasound findings

There was good agreement between neonatal cranial ultrasound diagnosis and visual assessment of MR images in the cases in which the ultrasound findings had been normal. This is consistent with the results of a study conducted by Roelants-van Rijn et al (2001), who compared neonatal ultrasound findings with MRI performed within the first four weeks of life and, in some cases, at term equivalent age. In contrast to the findings of this

study, Maalouf et al (2001) found that, in up to half of the infants with normal or mildly abnormal white matter on neonatal ultrasound, diffuse and excessive high signal intensity in white matter on MRI performed in the neonatal period was present. It is not known yet whether the finding of diffuse and excessive high signal intensity in white matter on neonatal MRI remains similar on MRI performed later in childhood/adolescence. Thus comparison of findings between the current study and the above mentioned study by Maalouf et al (2001) is limited. In addition, in these studies, the number of infants with normal ultrasound was small.

Agreement between neonatal ultrasound and visual assessment of MRI performed at the age 9-13 years was less good for non-parenchymal lesions. Fifty seven percent of the children with non-parenchymal lesions on neonatal ultrasound had abnormal findings on MRI, whereas 43% had normal MRI on visual inspection. This is consistent with previous studies on the comparison of neonatal ultrasound and MRI performed in the neonatal period that indicate that for non-parenchymal lesions, ultrasound has limited predictive value for lesions identified on visual analysis of MR images (Roelants-van Rijn et al, 2001; Debillon et al, 2003). However, in contrast, in the study conducted by Maalouf et al (2001), cranial ultrasound has been shown to be a good predictor for GLH and IVH (both are non-parenchymal lesions) on early MRI. In autopsy studies comparing ultrasound findings and neuropathology findings, the sensitivity of ultrasound for detecting lesions such as IVH and GLH varies from 50% (Pape et al, 1983) to up to 90% (Trounce, Fagan and Levene, 1986).

In the group of children with parenchymal lesions on neonatal ultrasound the agreement with MRI was good. In 88% of those with parenchymal lesions on ultrasound, abnormalities on MRI were detected on visual inspection. This is consistent with previous studies, e.g. deVries et al (1993), who found good agreement between the presence of parenchymal lesions and MRI at the age 11-32 months of age.

In the current study, in the group with parenchymal lesions on ultrasound, two children were diagnosed by visual inspection of MR images with an MCA infarct and one child with schizencephaly. Diagnosing arterial infarcts and/or neuronal migration disorders accurately

may be difficult with neonatal cranial ultrasound. In the two children with arterial infarct, parenchymal periventricular lesions had been seen on neonatal ultrasound but no diagnosis of an infarct had been made. Golomb et al (2003) have found ultrasound to be of low diagnostic value for detection of arterial infarcts in term neonates. This is in contrast with the findings of Cowan et al (2005), who compared ultrasound and MR imaging in 47 infants with MCA infarcts diagnosed by MRI. In over 60% of the children in their study, ultrasound showed evidence suggestive of infarction on early ultrasound (day 1-3) and in over 80% on late ultrasound (after day 4). However, in only 27% of the infants, the typical signs of abnormalities that had been reported by deVries et al (1997) to be diagnostic of a middle cerebral artery infarction were seen.

In the child with schizencephaly, a left sided parenchymal lesion was seen on neonatal ultrasound. Although not impossible, it may be difficult to identify the cleft and the adjacent polymicorgyria on ultrasound and usually the diagnosis is confirmed with MRI (Govaert and deVries, 1997).

7.7.5 Conclusions

Visual analysis of MR images obtained at age 9-13 years identified additional structural brain abnormalities that had not been diagnosed by neonatal cranial cranial ultrasound in a number of cases. In addition, those lesions that had been seen on neonatal ultrasound were characterised in more detail by MRI. The patterns identified on MRI in this selected group of preterm infants are similar to those described in the existing literature, even when comparing the findings to those of studies that investigated unselected groups of preterm infants and children. However, based on the results of histopathological studies it is likely that, in addition to lesions that are identifiable by visual inspection of MR images, subtle structural brain abnormalities are present in preterm children and that these may form the anatomical correlates of some of the neurological and neurodevelopmental impairments. Therefore, a quantitative MRI analysis method for detection of subtle brain abnormalities (voxel-based morphometry, VBM) has been used in the current study in addition to purely

visual inspection of MR images. The application of this method and the results obtained from the analyses are presented in the next chapter.

Chapter 8: Detection of subtle grey matter abnormalities by quantitative analysis of magnetic resonance imaging data using voxel-based morphometry

The main aim of this chapter is to investigate whether subtle grey matter abnormalities that may be present in the study population (but are not identified by visual inspection of MR images) can be detected by using voxel-based morphometry (VBM).

First, the basic theory of VBM using statistical parametric mapping (SPM) is outlined. The necessary processing steps that have to be performed before data can be entered in the statistical analysis are described, followed by a discussion of statistical concepts of SPM. Second, the application of VBM in the context of this study is described in detail, followed by presentation of the results of the single subject VBM analyses and discussion of the findings. This chapter focuses on the description of the VBM findings in the whole study population. Associations between these findings and outcome are the subject of subsequent chapters.

8.1 Voxel-based morphometry – basic theory

In this study, voxel-based morphometry (VBM) was used to quantitatively assess the MR images, with the aim of identifying structural abnormalities that may be too subtle to be detected by purely visual analysis of the images. VBM can characterise grey and white matter differences in structural MR scans. It allows for comprehensive identification of differences, not just in specific structures, but throughout the entire brain. It has been shown in a number of studies (see chapter 3 and chapter 4) to identify subtle brain abnormalities that are not detectable on visual analysis.

VBM measures differences in local densities of grey or white matter of the brain between different MR data sets (i.e. comparison of images from a group of patients or a single subject with images from a control group) through a voxel-wise comparison of multiple brain images. Comparison is made by using statistical parametric mapping to identify regional differences and finally make inferences about regional differences, i.e. p-values are assigned to specific regional differences. The stages of analysis include a number of pre-processing steps before statistical analyses can be carried out and these pre-processing steps are described in the following section in detail.

8.1.1 Pre-processing of 3D magnetic resonance imaging datasets

8.1.1.1 Spatial normalisation

To facilitate voxel based analysis of MR images, data from different subjects must derive from homologous parts of the brain. Therefore images must be put in the same stereo-tactic space. This is achieved by applying spatial transformations that make all images conform to a standardised brain (template), i.e. transform an image into a standard anatomical space. SPM99 minimises the sum of the squared differences between the image to be normalised and the template whilst maximizing the prior probability of the transformation. The maximum a posteriori solution is found iteratively: the algorithm starts with an initial parameter estimate and searches from there. The algorithm stops when the criterion is achieved (when the weighted sum of square differences no longer decreases or after a finite number of iterations). The process is divided into two components: linear (affine) and non-linear transformations. Affine transformations account for differences in position, orientation, and overall brain size. The subsequent non-linear transformation accounts for low spatial frequency and global variability in head shape.

8.1.1.2 Segmentation

Following spatial normalisation, images are segmented into three broad tissue types. Each voxel is classified as white matter, grey matter, or cerebro-spinal fluid. The classification is based on the intensity of the voxel combined with the probability of that voxel location being white matter, grey matter, or CSF based on prior probability maps (the prior probability maps have been derived from the template image originating from the Montreal Neurological Institute (MNI), which has been created from T1 weighted imaged of 152 healthy adult subjects). To obtain prior probability maps, original images were segmented into binary images of grey matter, white matter and cerebro-spinal fluid, and then normalised. The resulting probability maps are means of these binary images, so they contain values between zero and one. They present the a priori probability of a voxel being either grey matter, or white matter, or cerebro-spinal fluid, after an image has been normalised to a template of the same space.

8.1.1.3 Spatial smoothing

The image is then smoothed using a Gaussian kernel, which gives a weighted average of tissue density in a defined area surrounding the selected voxel. Smoothing has several main objectives. It increases signal relative to noise and, after smoothing, the data conform more closely to the Gaussian field model. It also sensitises the data analysis to the specific spatial scale corresponding to the full width at half maximum (FWHM, width of the kernel at half of the maximum of the height of the Gaussian) of the smoothed data.

Figure 8.1 below summarises the pre-processing steps that are necessary before data can be entered into statistical analysis.

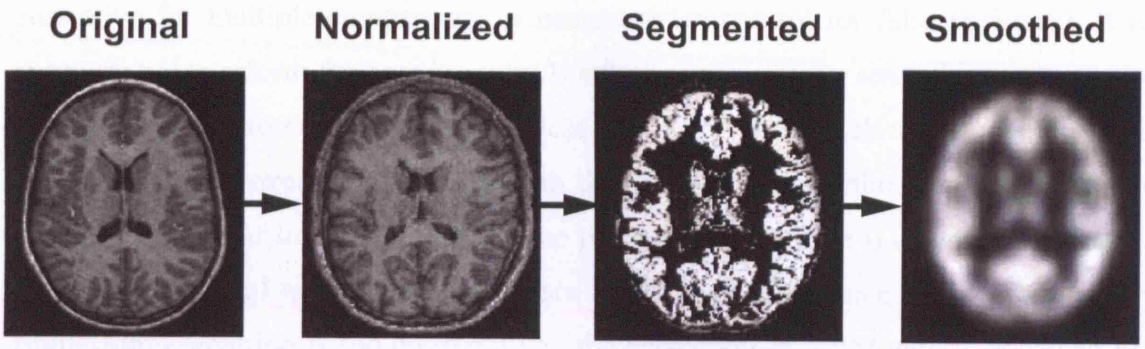


Figure 8.1: Pre-Processing steps in voxel-based-morphometry

8.1.2 Statistical analysis of MRI data using voxel-based morphometry

After pre-processing of the images, statistical analysis can be conducted in order to identify differences in local densities of grey matter or white matter between groups (or between an individual and a group). The output of this is a statistical map that displays regions in which there is a significant difference in the density of grey matter or white matter between compared groups.

For the analysis of regionally specific differences the General Linear Model (GLM) is used. The GLM is an equation that relates what is observed, to what was expected to be observed, by expressing the observations (response variable) as a linear combination of expected components (explanatory variables) and some residual error. The concept of GLMs places many commonly used models (e.g. simple t-test, multiple linear regression, or analysis of covariance) in a unified framework. In VBM, a t-test is carried out on each voxel. The output is a three dimensional statistical image ("map") formed of thousands of t-tests, with a t-value for each voxel (SPM{t}). SPM{t} is then transformed to Z scores. The Z scores are a way that is used in SPM to display and analyse the p-values from the t statistics. They are the numbers from the unit normal distribution (mean 0 sd/variance 1) that would give

the same p value as the t-statistics. (In the versions of SPM from SPM99 onwards, t-statistics rather than Z scores are used). Since these SPMs contain a huge number of voxels, and a separate t-test is performed for each voxel, the maps are not directly interpretable and correction for multiple comparisons is necessary (to control for false positives). It is not appropriate to perform the correction by Bonferroni procedures, since this assumes that the multiple tests performed were independent. However, the voxels are spatially correlated (i.e. data in one voxel are correlated with the data from a neighbouring voxel) due to the initial resolution of images and due to the processing procedure (i.e. smoothing), and as a result the statistical tests at each voxel are not independent. Since in these circumstances Bonferroni correction is too conservative, the correction in VBM can be achieved in two principle ways: First, by applying the Gaussian Field Theory (or Random Field Theory, RFT). It can be regarded akin to a Bonferroni correction, using RESELS (rather than voxels). where the number of RESELS is similar to the number of independent observations in the image (a RESEL is a "resolution element", defined as a block of pixels of the same size as the FWHM of the smoothness of the image). RFT determines how many RESELS are in an image, uses RESEL count and calculation to get the expected Euler characteristics (which is a geometric measure that counts the number of connected components minus the number of "holes" in a volume of the image. It is a property of the image after thresholding, and a good approximation to the probability of observing one or more significant regions at a given threshold. An estimation of the Euler characteristics at any given threshold is possible if the number of RESELS is known). The Euler characteristics can be thought of as the number of blobs of Z scores above a certain threshold. RFT also uses the Euler characteristics to give a correct threshold for the required false positive rate

The obtained Z value (or alternatively the t-statistics) in a specific location in the SPM maps can be used uncorrected for multiple comparisons if there was an a priori anatomically defined hypothesis about differences in those specific voxels. However, if there is no a priori hypothesis, the correction for multiple non-independent comparisons is necessary. Gaussian Field Theory allows statistical inferences to be made (i.e. compute threshold p-values for appropriate Family Wise Error (FWE) rate). Correction can either be

over the entire brain (in the absence of a prior hypothesis), or corrected over small volumes if priori hypotheses exist for specific brain regions (note that hypotheses are required to be at the voxel level for uncorrected statistics to be valid). This approach controls the probability of reporting a false positive anywhere in the brain (which is needed when a conclusion that is drawn from the various individual inferences will be wrong if at least one of them is). However, in some circumstances this is too conservative (when it is important to consider the number of erroneous rejections and not only whether *any error* was made). In this case, there is a second way that allows drawing conclusions form the individual inferences by using False Discovery Rate (FDR). FDR is a new approach to the multiple comparisons problem. Instead of controlling the chance of *any* false positives (as Bonferroni or Random Field methods do), FDR controls the expected *proportion* of false positives among supra-threshold voxels (i.e. among those found to be positive).

In terms of application of the above discussed concepts in SPM, it is generally the case that the type of questions asked of the data when using SPM requires FWE correction to be used, and FDR is not appropriate. In particular, one usually wants to know whether any individual region (e.g. in VBM a difference in density of white or grey matter) is statistically reliable, for which FDR does not provide the required control. However, FDR can provide the appropriate control if the question that is investigated is "are *any* regions different on VBM", provided that the data are not discussed in terms of individual regions that have been found to be significant.

8.1.3 Display of results

Results can be displayed by superimposition of the significant regions on to a "glass brain" (see figure 8.2 below), an image created on the mean of the normalised images (see figure 8.3 below), or on the normalised image of an individual's brain. The glass brain displays all voxels above a defined threshold (e.g. $p<0.05$), and superimposing the significant voxels on a mean image allows presentation of the anatomical location of the peaks. In these presentations, the Z scores obtained from the statistical testing are indicated by the colour

according to the scale as shown in figure 8.3. Note that in SPM images are displayed in neurological convention, i.e. left=left and right=right.

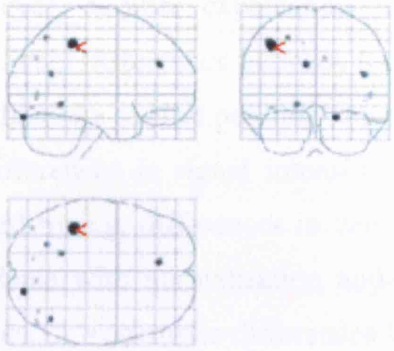


Figure 8.2: Example of superimposition of significant grey matter regions on each of three projections (sagittal, coronal, axial) of a 3D "glass brain"

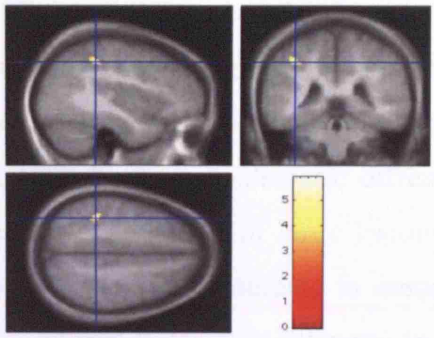


Figure 8.3: Example of superimposition of a significant grey matter region on individual sagittal, coronal and axial images from a mean 3D dataset. The blue cross indicates the voxel of interest for selecting the relevant image positions. This position can be selected using the red cursor shown in fig 8.2

8.1.4 Interpretation of results

The most general interpretation of significant results is that there is a difference in density in white or grey matter in the pre-processed images between two groups of subjects (or between a single subject and a control group) in a particular region, which, in the case of grey matter, might be due to differences in neutropil, neuronal size, axonal or dendritic arborisation. However, it is not entirely clear yet whether VBM-detected differences are actually related to these tissue characteristics. Alternatively, VBM-detected differences might be caused by other factors. For example, a decrease in density can be interpreted as a structure being smaller in one group than the other (e.g. thinning of the cortex, atrophy of subcortical grey matter structures). An increase in density can be interpreted as a true

increase in grey matter (abnormal distribution of grey matter, e.g. subtle focal cortical dysplasia) or abnormalities of gyral pattern (e.g. deeper gyri).

However, another explanation for VBM-detected differences is that there are other significant differences between the datasets of the compared groups in a particular region, and there are several possible reasons for this. Pre-processing sensitises the test to detection of differences in signal intensities between datasets. Thus systematic differences can be caused by e.g. differences in ventricular size or the presence of large lesions resulting in problems with normalisation and/or segmentation, motion artefacts in some of the MR images, or systematic differences between the groups in contrast intensity in the MR data resulting in problems with segmentations and possibly misclassification of tissue. These and other reasons may result in differences that are detectable by VBM. All these differences are real, but they may not necessarily be due to true (anatomical or biological) differences in grey or white matter density between groups. (A discussion of the possible interpretations of increases and decreases in grey matter density found in the population of the current study is given below in the individual sections that report the findings, i.e. sections 8.3.1.1, 8.3.2.1.1, 8.3.2.2.1, 8.3.2.3.1, 8.3.2.4.1, and, in addition, a more overall discussion is provided in section 8.4.1).

8.2 Detection of subtle grey matter abnormalities in preterm children with epilepsy and/or cognitive impairment - Methods

MRI data from 51 preterm children (22 with epilepsy, 29 without epilepsy) of the available 54 MRI datasets were included. In three cases (AP, TS, JC) no 3D datasets were available since the MRIs had been performed elsewhere. Six of the 51 datasets had to be excluded since pre-processing was unsuccessful (see section 8.2.1 below).

As described in chapter 4, section 4.4.1, 3D structural MR images were acquired using a T1 weighted 3D MPRAGE sequence. The data were processed and analysed in SPM99 (Wellcome Department of Cognitive Neurology, London, UK).

VBM was initially developed to examine differences in brain structure between groups of adult subjects. In this study, VBM is being used to examine structural abnormalities in individual paediatric brains, some of which are likely to have focal lesions. This has some implications for pre-processing and statistical analysis of the imaging data, which are described in the following sections.

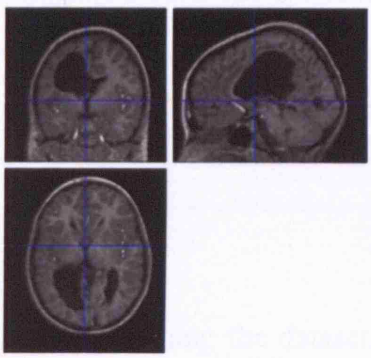
8.2.1 Pre-processing of 3D datasets

In the current study, a number of approaches were attempted to optimise normalisation and segmentation, including disabling the SPM implicit brain mask and, in some datasets, masking of lesions.

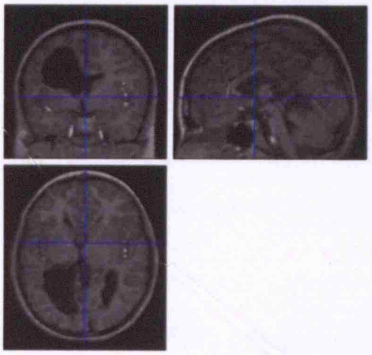
Disabling the implicit brain mask: All datasets were normalised to the MNI template using the implicit brain mask in SPM/VBM (which excludes information from structures outside the brain in the normalisation process) and then compared to datasets that had been normalised without using the implicit brain mask. In images without lesions, no significant difference in the normalisation results was seen. In subjects with lesions, however, disabling the implicit brain mask (i.e. including information from structures outside the brain in the normalisation process) gave better normalisation results. It was therefore decided to disable the implicit brain mask as a default for normalisation.

Masking of lesions: Normalisation involves minimising the sum of squared differences between the two images following affine and non-linear spatial deformations of one of the datasets. In subjects with brain lesions that were sufficiently large to cause the standard normalisation to the template to fail, the lesions were masked using in-house written software. This procedure required manual delineation of the lesions. By masking areas of the brain, information from these (abnormal) areas is not being included when calculating the optimal normalisation parameters, and this may improve the normalisation process (in the later processing steps and analysis, the whole brain is included once again). In some

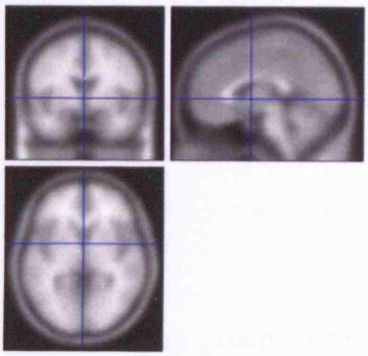
subjects, different sizes of lesion masks were tried, which did not noticeably affect the normalisation result. Application of this masking procedure resulted in an improved normalisation result in 8 of the 11 datasets in which this procedure was applied (LO, NK, CT, KS, SHay, ML, EG, PP, LR, JW, HJ, see appendix 7) and in successful normalisation in seven datasets (example see figures 8.4 a-c). In two datasets in which the use of lesion masks led to a satisfying normalisation result, the subsequent segmentation failed. In total, six datasets were omitted from VBM analysis. In all six cases visual analysis of MR images showed severe white matter reduction, which was combined with visible grey matter abnormalities in all cases (cortical lesions n=3, hippocampal abnormalities n=5, basal ganglia/thalamus abnormalities n= 2). Five of these six children had epilepsy.



a) Normalisation without lesion masked. Note that image dimensions, shape and position do not match those of the template



b) Normalisation with lesion masked, showing greatly improved correspondence to the template



c) Template

Figures 8.4 a-c:

8.2.2 Grey and white matter age dependence

Previous studies (for review see Sowell, Thompson and Toga, 2004) have shown that there are age-related changes of grey and white matter in infancy and childhood. It is important to take this into account when anatomical changes are being investigated. For this study, VBM analyses were performed to determine a possible age dependency among the age range of the age matched controls/patients in this current study. The control group of 16 neurologically normal subjects (age range 84–180 months, median 145 months) was divided into three age groups as shown in table 8.1 below.

Table 8.1: Age and gender distribution in the three groups created from the group of 16 neurologically normal subjects for investigation of age related effects on grey and white matter changes

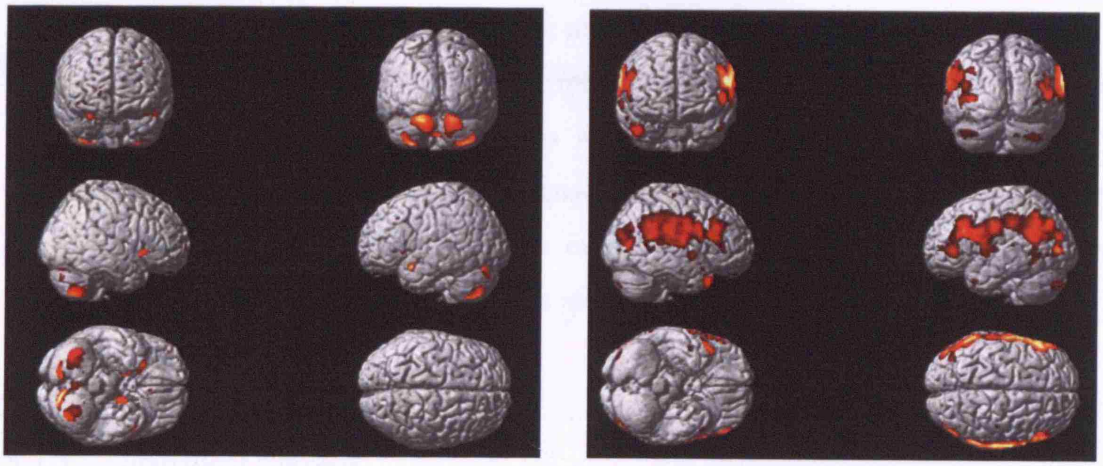
	n	**Age (months)**	**Male/female**
Group 1	6	>= 84- <132	4/2
Group 2	5	>=132- <156	3/2
Group 3	5	>=156- 180	2/3

After smoothing the datasets to 12mm FHMW, t-tests between the three age groups were performed to investigate whether there were age related grey and white matter differences. In order to account for any non-linearity of age related changes, age was entered as a categorical variable (creating dummy variables for age group 1, group 2, and group 3 respectively) rather than a continuous variable. Since it was of interest to investigate whether there was *any difference* between the groups, the resultant t-statistic maps were thresholded at a p-value of <0.05 corrected for multiple comparisons using the False Discovery Rate (FDR) rather than Family Wise Error (FWE).

There were no significant differences in white matter between the three groups. With regard to age related grey matter changes there were significant differences between the age groups. These differences were most pronounced between the youngest (group 1) and the oldest age group (group 3). Figures 8.5 a and 8.5 b below display the results of the VBM

analysis for grey matter differences between group 1 and group 3, superimposed on the surface of the brain. Based on these results it was decided to enter age as a categorical covariate in the statistical analyses comparing each individual dataset of the preterm children with the group of term born neurologically normal controls.

Figures 8.5 a and b: VBM results from the age comparison (group 1 versus group 3) of grey matter at $p<0.05$ corrected (FDR), displayed at a corrected threshold of $p=0.05$. Images are displayed in neurological convention (left =left, right=right)



a) Group1 less grey matter than group 3 b) Group1 more grey matter than group 3

8.2.3 Effects of gender

Sex related differences in white and grey matter have been described in many studies using VBM (e.g. Good et al, 2001). However, since gender was relatively well balanced in the control group (7 female, 9 male), this was not entered as a covariate in the VBM analysis.

8.2.4 Single subject analysis

As mentioned above, the parametric statistical tests are carried out within the framework of the General Linear Model. In order for these tests to be valid, the errors must be identically and normally distributed. In parametric statistics it is assumed that the group difference is normally distributed. However, when one of the groups has only one subject, the difference may be highly non-normal and the distribution of the ensuing statistic will not conform to parametric assumptions. Salmond et al (2002) have established that group size (i.e. the design) may influence the robustness to violations of normality at low levels of smoothing. The results of their study suggest that the minimal amount of smoothing for single subject versus group comparison design is 12 mm FHMW. When the smoothing kernel was reduced to 8mm or 4 mm, the analyses were significantly prone to false positives. In contrast, there was no evidence that the analyses were invalid when a smoothing kernel of 12 mm was applied. Therefore, for this current study, in which comparison between a single subject and a group of controls was made, data were smoothed to 12 mm.

8.2.5 Statistical analysis

As described above, images were normalised to the standard MNI template, and then segmented into grey matter, white matter and cerebrospinal fluid (CSF). Grey matter segments were entered into statistical analysis after smoothing to a 12 mm Gaussian kernel. Each patient's dataset was compared to 16 control datasets (single subject-group comparison) and age was entered as a categorical covariate to account for a possible non-linear age dependency.

Inferences from statistical maps were made by using significance values corrected for multiple comparisons using Family Wise Error (FWE). P-values were used as a measure of an effect rather than strict hypothesis testing. Therefore corrected p-values of <0.1 were deemed as suggestive of evidence for grey matter abnormalities in the subject

Anatomical location of an abnormality was identified by using the anatomical brain atlas by Duvernoy (1991). The statistical parametric maps were superimposed on the individual subject's normalised image in order to help identify the anatomical location. In some cases the SPMs were also superimposed on a mean image created from the normalised datasets of the controls. This additional method of display of results was chosen for subjects in whom there was evidence that controls might have more grey matter and the abnormalities detected exceed the patient's data extent (i.e. if the peaks appeared to be outside the patient's brain).The maps are displayed at p=0.0001 uncorrected. Images are displayed in neurological convention (left=left, right=right), and the crosshairs show the location of the maximal peak. In some subjects, in whom multiple peaks were identified, only one fairly representative example of superimposed peaks was chosen for illustration.

Figure 8.6 below summarises the approach to pre-processing and statistical analyses of the 3D MPRAGE datasets.

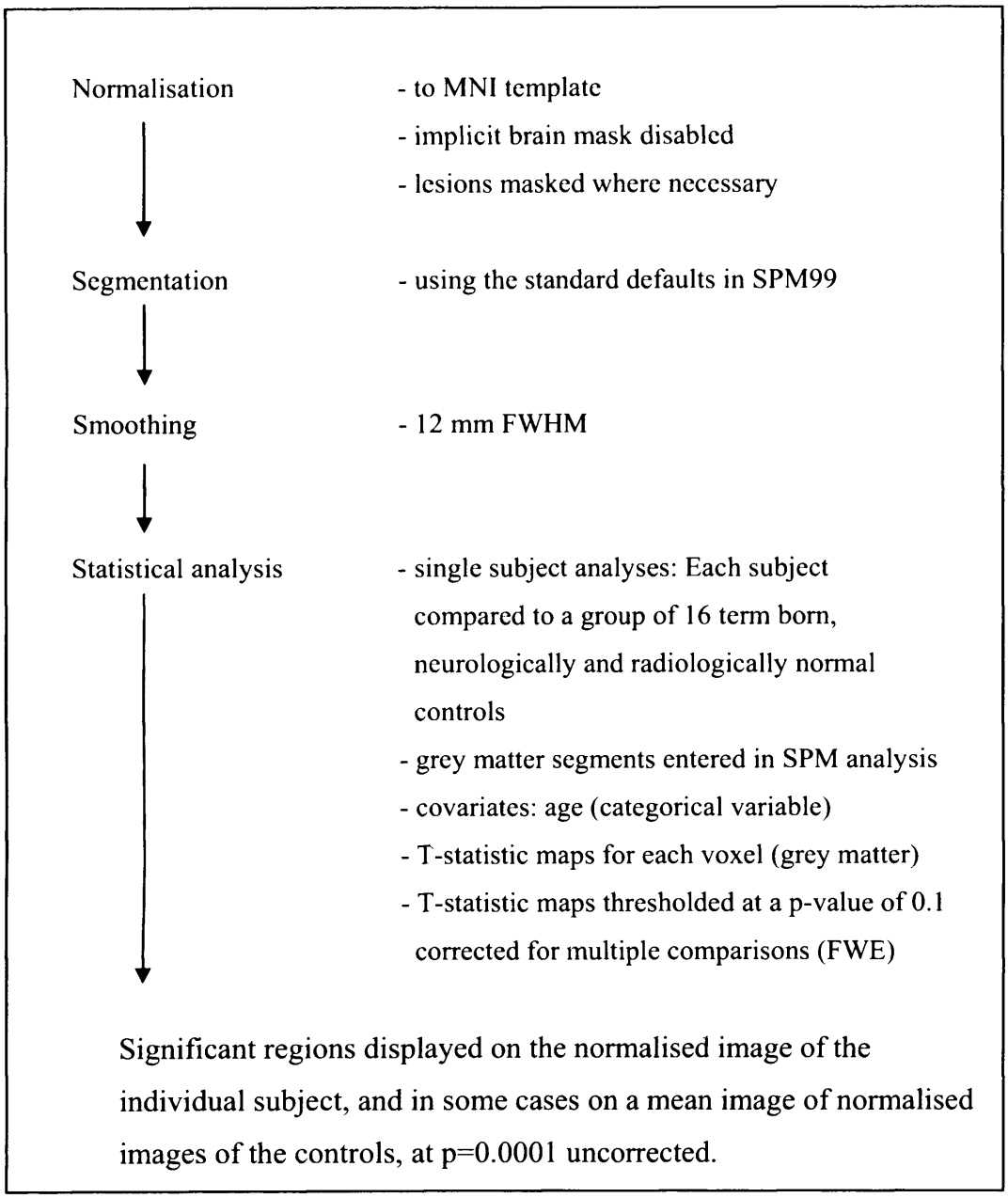


Figure 8.6: Summary of the pre-processing steps and statistical analysis of 3D MPRAGE datasets

8.2.6 Classification of VBM results

VBM results were classified as "no abnormalities" (i.e. the VBM analysis did not detect a difference in grey matter density between a dataset of a preterm child and the datasets of the VBM control group), "focal abnormalities" when one or two peaks in one or different regions/structures of the brain or multiple peaks within one cluster/confined structure (e.g. basal ganglia) were detected, and as "widespread" abnormalities consisting of three or more peaks that were not within one cluster/confined structure. This classification was chosen since some of the images already showed brain abnormalities on visual inspection, and multiple peaks in VBM were therefore regarded as a surrogate for more widespread damage (although this may have been reported in the VBM analyses as multiple focal abnormalities).

8.3 Results

Forty five (from 17 children with epilepsy and from 28 children without epilepsy) of the original 51 available 3D MPRAGE datasets were entered in the VBM analysis and individually compared to the VBM control group.

In this section, first, the overall results of the VBM analysis and associations between the VBM findings and findings from the visual inspection of MR images are presented for the whole group (table 8.2). The VBM findings for each child are shown in table 8.3 along with a summary of the results from the visual inspection, neurological status, information on presence or absence of epilepsy and IQ scores. Second, the VBM findings are presented in more detail for those with normal and abnormal MRI on visual inspection and, in addition, within the individual MR categories. The subgroup with periventricular white matter reduction is of particular interest in the context of this thesis and the VBM results of this subgroup are presented in more detail in section 8.3.2.3. Following this is a section (8.3.2.4) presenting the VBM findings in the thalami and the basal ganglia. At the end of each of these sections, possible interpretations of the VBM findings described in this

section are discussed. It was decided to include these short discussions on some aspects of the VBM results into the individual results sections rather than in the overall discussion for easier reference. A more general discussion of methodological issues that may affect VBM findings is included in the overall discussion at the end of this chapter.

Table 8.2 below summarises the findings from VBM grey matter analysis according to the categories used for classification of MRI findings on visual inspection of the images. In 17 (38%) of the 45 datasets no differences in grey matter density between the individual preterm datasets and the VBM control group were detected by VBM. Nine of these 17 datasets had been judged as normal on visual inspection.

In 28 (62%) datasets VBM analysis detected grey matter differences between the individual datasets and the VBM control group. In 16 (57%) of these 28 datasets focal VBM abnormalities were seen (i.e. 1-2 peaks). In 12 (43%) of the datasets more widespread grey matter abnormalities were detected (i.e. >=3 peaks).

In those with normal MRI on visual inspection, the VBM detected abnormalities were focal except in one case (see table 8.2 and 8.3). In contrast, in those with abnormal MRI on visual inspection, only 30 % had focal VBM abnormalities and 70% had more widespread VBM abnormalities. On statistical testing there was no evidence for a significant association between normal or abnormal MRI on visual inspection and the presence or absence of VBM grey matter abnormalities (Chi-square test, p=0.22) (see section 8.4.4.2 for discussion). However, the association between normal or abnormal MRI on visual inspection and the number of detected VBM grey matter abnormalities categorised as none, 1-2, or >=3, was significant (Chi-square test, linear-by-linear association, p=0.04).

Furthermore, there was evidence for a significant association between the presence or absence of periventricular white matter abnormalities identified on visual inspection and the number of VBM detected grey matter abnormalities (Chi-square test, linear-by-linear association, p=0.02). In addition, statistical testing indicated that there was a significant correlation between the degree of white matter reduction and the number of VBM-detected

grey matter abnormalities (Spearman's ρ=0.6, p=0.04) indicating that widespread (subtle) grey matter injury is likely to be present in the children with more severe degrees of white matter reduction.

The presence of visible grey matter abnormalities was not significantly associated with the number of VBM-detected grey matter abnormalities (Chi-square test, linear-by-linear association, p=0.2). Similarly, there was no evidence on statistical testing that the presence or absence of a combination of white and grey matter lesions identified on visual inspection was significantly associated with number of VBM detected grey matter abnormalities (Fisher's Exact test, p=0.16).

Irrespective of whether the visual inspection of the MRI was normal or abnormal, or whether focal or widespread VBM were detected, the most frequent locations in which VBM detected grey matter differences between the individual preterm datasets and the VBM control group were the temporal lobe (n=15), parietal lobe (n=12), the thalami (n=12), frontal lobe (n=10) and the cerebellum (n=8). Abnormalities were also seen in the cingulate cortex (n=5), basal ganglia (n=3), hippocampi (n=3), the insular cortex (n=2) and the occipital lobe (n=1), but far less frequently.

Table 8.2: Summary of findings from VBM grey matter analysis and findings on visual inspection of MR images. For VBM analysis, each dataset from a preterm children was compared to the VBM control group

MRI visual inspection **(n=45 datasets)**	**No abnormalities on VBM** **n=17**	**Focal abnormalities on VBM (1-2)** **n=16**	**Widespread abnormalities on VBM (>=3)** **n=12**
MRI visual inspection normal (n=18)	9	8	1
MRI visual inspection abnormal# (n=27)	8	8	11
MRI categories*:			
MRI white matter abnormality* (n=24)	7	6	11
- Gliosis only (n=5)	3	2	-
- White matter reduction (n=19)	4	4	11
Mild/moderate *(n= 9)*	*3*	*3*	*3*
Severe *(n=10)*	*1*	*1*	*8*
Isolated grey matter abnormality (n=3)	1	2	-
Combination of white and grey matter abnormalities (n=10)	2	3	5

* Categories are not mutually exclusive

Any abnormality identified on visual inspection of MR images

Table 8.3 on the following pages shows for each child the detailed VBM findings alongside a summary of the results from visual inspection of MR images, information on neurological status, presence or absence of epilepsy and IQ scores.

Table 8.3: Details of VBM grey matter findings, information on presence or absence of epilepsy, neurological status, and IQ scores for each preterm child (n=45)

	Epilepsy	FS IQ PIQ VIQ	Neurology	Visual inspection of MRI	VBM Number of peaks (0, 1-2, >=3)	VBM Location of peaks	Co-ordinates (MNI) X Y Z	More/less gm in subject than in controls; p-value (corrected)
TR	+	96 78 111	normal	normal	0	-	-	-
TO	+	85 97 77	normal	normal	0	-	-	-
RK	-	112 96 123	normal	normal	0	-	-	-
DS	-	123 109 131	suspicious	normal	0	-	-	-
JoCol	-	107 99 113	suspicious	normal	0	-	-	-
AC	-	93 85 103	normal	normal	0	-	-	-
VH	-	102 118 90	normal	normal	0	-	-	-
CR (I)	-	88 74 103	suspicious	normal	0	-	-	-
JSk	-	109 102 113	normal	normal	0	-	-	-
AT	-	60 54 72	suspicious	HC bilateral small	0	-	-	-
MC	-	59 54 68	suspicious	MWMR bilateral (po)	0	-	-	-
LiWi	-	103 100	suspicious	Periv. gliosis bilateral	0	-	-	-

		107		(right>left, po, f,)				
LWa	-	100 96 102	normal	Periv. gliosis (po)	0	-	-	-
NSi	-	71 57 87	abnormal	Periv. gliosis bilateral (po, f)	0	-	-	-
SD	+	86 95 80	suspicious	MWMR left (po) HC bilateral small	0	-	-	-
LH	-	108 96 117	abnormal	MWMR bilateral (left>right, cs) Gliosis (cs)	0	-	-	-
RR (II)	-	98 101 96	abnormal	SWMR (left, cs) BG, Thal	0	-	-	-
GO ***Fig 8.20***	-	108 99 115	normal	Gliosis bilateral (right>left, cs) BG, Thal	1-2	Cerebellum left	-18 -51 -58	Less grey matter: P=0.048
TM ***Fig 8.8***	+	No data	normal	normal	1-2	Insular gyrus left	-39 -22 14	Less grey matter: P=0.054
SSk ***Fig 8.9***	+	106 92 117	suspicious	normal	1-2	Cingulate gyrus left	-20 -42 36	More grey matter: P=0.088
LB ***Fig 8.10***	+	64 61 72	suspicious	MWMR bilateral (po) HC bilateral small	1-2	Hippocampus left	-16 -9 -18	Less grey matter: P=0.024
BK ***Fig 8.12***	+; NNS	63 70 60	suspicious	BG (caudate right)	1-2	Superior temporal gyrus right	62 -50 18	Less grey matter: P=0.017
TC ***Fig 8.11***	+	92 96 90	normal	MWMR bilateral (cs)	1-2	Superior parietal gyrus right	26 -66 51	Less grey matter: P=0.072

SH ***Fig 8.14***	+	96 86 106	abnormal	SWMR bilateral (left>right, po) Gliosis (po)	1-2	Cingulate gyrus right Cingulate gyrus left	22 -38 40 -21 -40 36	More grey matter: P=0.066 P=0.089
CS ***Fig 8.15***	-	114 107 117	suspicious	normal	1-2	Inferior frontal gyrus left	-32 30 20	More grey matter: P=0.046
MD ***Fig 8.19***	-	105 104 106	normal	normal	1-2	Medial orbital gyrus right	20 46 -21	More grey matter: P=0.067
ASa ***Fig 8.13***	-	105 109 102	normal	normal	1-2	Thalamus right Thalamus left	26 -27 4; 18 -14 8 -14 -8 4; -18 -16 9	More grey matter: P=0.000; p=0.008 P=0.000; p=0.033
SDaW ***Fig 8.16***	-	87 82 93	suspicious	normal	1-2	Superior temporal gyrus right	62 -50 18	Less grey matter: P=0.010
BA ***Fig 8.21***	-	96 94 99	normal	normal	1-2	Superior/middle temporal gyrus right	44 26 -40	Less grey matter: P=0.083
SF ***Fig 8.17***	-	87 88 89	normal	normal	1-2	Thalamus left	-14 -8 4	More grey matter: P=0.025
BBe ***Fig 8.18***	-	72 58 88	normal	HC small bilateral	1-2	Superior frontal gyrus right	24 26 48	Less grey matter: P=0.067
DSk ***Fig 8.22***	-	109 102 113	suspicious	MWMR bilateral (cs) Chiari I	1-2	Cerebellum left	-24 -58 -57	Less grey matter: P=0.095
NK ***Fig 8.23***	-	86 75 100	suspicious	Periv. gliosis (left, po) HC bilateral Cortical lesion	1-2	Temporal lobe right (superior temporal gyrus, site of shunt)	64 -48 0	Less grey matter: P=0.000

				temporal-parietal right (shunt)		Cingulate gyrus right	2 26 26	P=0.012
CO (II) ***Fig 8.35***	-	67 56 86	abnormal	SWMR bilateral (right>left, right:po,cs, f; left po,f)	>=3	Region of thalamus right Parahippocampal gyrus right Parietal lobe (region of parieto-occipital fissure) right	2 -14 4 28 -36 -4 15 -62 10	Less grey matter: P=0.002 P=0.004 P=0.011
PD ***Fig 8.30***	+	47 45 67	abnormal	MWMR bilateral (left>right,cs) Gliosis (cs) Cerebellum small bilateral	>=3	Thalamus right Thalamus left Cerebellum right Cerebellum left Temporal lobe (region of middle temporal gyrus) right	26 -27 4; 18 -9 6 -14 -8 4 9 -44 -57;46 -63 -40 -45 -51 -40;-10 -51 -56 64 -9 -21	More grey matter: P=0.000;P=0.080 P=0.057 Less grey matter: P=0.086; p=0.024 P=0.000; p=0.017 P=0.003
WS ***Fig 8.24***	+; NNS	74 45 105	abnormal	MWMR bilateral (left>right,po) Gliosis (po,cs)	>=3	Occipital lobe (near collateral sulcus) right Thalamus right Cerebellum left Frontal lobe (region of inferior frontal gyrus) right Postcentral gyrus right Parietal lobe (superior parietal) right	32 -66 2 26 -27 4 27 -82 -36 51 34 -2 56 -28 48 26 -66 51	More grey matter: P=0.012 P=0.019 Less grey matter: P=0.004 P=0.026 P=0.076 P=0.080

LR ***Fig 8.28***	+	59 52 70	suspicious	SWMR bilateral (right>left, po)	>=3			More grey matter:
						Thalamus right	15 -14 0	P=0.000
						Thalamus left	-14 -9 -2 (biggest cluster)	P=0.000
								Less grey matter:
						Region of putamen right	15 -34 -2 (biggest cluster)	P=0.000
						Parietal lobe (region of parieto-occipital fissure) right	14 -62 10	P=0.000
						Hippocampus right	27 -38 -4	P=0.001
						Temporal lobe (region of middle temporal gyrus) right	45 22 -40 (biggest cluster);51 16 -40	P=0.034;P=0.040
JW ***Fig 8.31***	+; NNS	-	abnormal	SWMR bilateral (po) Schizencephaly bilateral, adjacent polymicrogyria, HC small bilateral	>=3			More grey matter:
						Thalamus left	-14 -8 4	P=0.001
						Fronto-parietal right; (region of schizencephaly)	48 -10 30	P=0.001
						Thalamus right	16 -14 3	P=0.006
						Fronto-temporal left; (region of schizencephaly)	-33 30 20	P=0.007
						Frontal lobe left (adjacent to schizencephaly)	-28 42 -2	P=0.033
						Parietal lobe left	-48 -36 34;-50 -12 27	P=0.050;P=0.058
								Less grey matter:
						Temporal lobe right	26 -2 -36; 32 14 -38; 48 15 -32	P=0.000;P=0.000; P=0.000
						Temporal lobe left	-48 12 -34;-27 4 -28	P=0.000;P=0.000
						Frontal left (region of schizencephaly)	-21 27 -28	P=0.000
						Parietal lobe left	-58 -60 18;-50 -74 33	P=0.000;P=0.001
						Parietal lobe right	62 -50 18	P=0.001
						Fronto-partietal (region of schizencephaly)	12 0 66	P=0.001

						Cerebellum left Insula left	-20 -82 -34 -39 -24 14	P=0.023 P=0.036
AM ***Fig 8.27***	+; NNS	66 46 64	abnormal	SWMR right (po,cs) Gliosis (po, cs) Cortex parietal right	>=3	Parietal lobe (region of precentral gyrus) right Region of cingulate gyrus/sulcus right Temporo-parietal right (region of shunt)	21 -6 44 20 16 34 54 -2 45 (biggest cluster);64 -48 32	More grey matter: P=0.009 P=0.060 Less grey matter: P=0.011;P=0.043
ML ***Fig 8.34***	+; NNS	-	abnormal	SWMR bilateral (left > right, po,cs,f)) gliosis (po,cs) Cortex (MCA left) Cerebellum small HC left small	>=3	Thalamus left Thalamus right Temporal lobe left adj. to MCA region Parietal lobe left Cerebellum left Cerebellum right Temporal lobe left, MCA region Parietal lobe left (not adj. to MCA region) Amygdala/hippocampus left	-14 -9 2 14 -14 2 -42 -48 -3;-40 -26 -8 -40 -10 30;-48 -36 34; 42 -8 30 -15 -51 -57 15 -51 -56 -22 -50 -14 -46 -54 48;-34 -78 40; -57 -10 40 -18 -10 -20	More grey matter: P=0.000 P=0.001 P=0.000;P=0.001 P=0.007;P=0.029; P=0.035 Less grey matter: P=0.000 P=0.000 P=0.000 P=0.000;P=0.000; P=0.002 P=0.051
AU ***Fig 8.32***	+	-	abnormal	SWMR bilateral (po,cs,f) HC abnormal signal right	>=3	Thalamus right Thalamus left Temporal lobe (region of superior temporal gyrus) right Frontal lobe (region of	26 -27 4; 20 -20 3 -14 -8 4 62 -50 18; 64 -45 24; 66 -34 27 -48 9 45	More grey matter: P=0.000; p=0.016 P=0.002 Less grey matter: P=0.000; p=0.0001; p=0.0002 P=0.019

						middle frontal gyrus left Cingulate gyrus left	 -50 -75 32	 P=0.030
EF ***Fig 8.33***	+	-	abnormal	SWMR bilateral (left >right, po,cs,f) Gliosis (po,cs)	>=3	 Frontal lobe (region of inferior frontal gyrus left Temporal lobe (region of superior temporal gyrus) right Parietal lobe bilateral Cerebellum left Interhemispheric fissure	 -33 28 21 68 14 -2 46 -27 62; -51 -72 30 -10 -51 -56 -50 3 3	More grey matter: P=0.044 P=0.049 Less grey matter: P=0.010;p=0.089 P=0.011 P=0.022
JRu ***Fig 8.29***	-	84 76 95	normal	normal	>=3	 Thalamus/Gl pallidus/putamen right Thalamus left Angular gyrus left Temporal lobe right (region of superior temporal gyrus ; middle temporal gyrus) Temporal lobe (middle temporal gyrus) left	 15 -9 0 (biggest cluster);26 -27 4 -14 -9 2 -50 -74 33 (biggest cluster) 62 -50 18 45 22 -40 (biggest cluster) -51 10 -33	More grey matter: P=0.000;P=0.000 P=0.000 Less grey matter: P=0.005 P=0.009 p=0.024 p=0.010
DHay ***Fig 8.25***	-	116 109 119	suspicious	MWMR bilateral (po)	>=3	 Thalamus right Thalamus left Supramarginal gyrus right Intraparietal sulcus right Frontal lobe (region of middle frontal sulcus) right Precentral gyrus right Parietal lobe (region of intraparietal sulcus left)	 26 -27 4; 12 -14 -2 -14 -9 2 (biggest cluster) 52 -45 46 (biggest cluster) 39 -68 48 20 36 45 (biggest cluster) 56 -3 42 -34 -78 40 (biggest cluster)	More grey matter: P=0.003;0.006 P=0.000 Less grey matter: P=0.001 P=0.023 P=0.069 P=0.047 P=0.019

LO(I) ***Fig 8.26***	-	88 74 103	suspicious	SWMR right (po,cs), Cortex left parietal (shunt) HC small left Cerebellum small	>=3	Thalamus right	26 -27 6 (biggest cluster)	More grey matter: P=0.009
						Frontal lobe (region of middle frontal gyrus) right	48 46 2 (biggest cluster)	P=0.025
						Frontal lobe right	30 20 -20	P=0.027
						Caudate left	-24 -28 15 (biggest cluster)	P=0.031
						Frontal lobe (region of middle frontal gyrus) left	-42 -48 3;-44 50 -2	P=0.039;P=0.080
								Less grey matter:
						Parietal lobe(region of shunt) left	-34 -78 40	P=0.000
						Temporo-parietal right	64 -39 9 (biggest cluster)	p=0.000
						Temporal lobe (region of enlarged ventricle) right	21 -58 -12	P=0.000
						Temporo-parietal left	-52 -70 3	P=0.000
						Cerebellum left	-22 -82 -40	P=0.077

Neurology: ***normal:*** entirely normal neurological examination; ***suspicious:*** non-specific signs like asymmetry of muscular tone or reflexes, muscular hypotonia, or muscular hypertonia (but without definite signs of spastic cerebral palsy); ***abnormal:*** clear neurological signs of spastic cerebral palsy (increased muscular tone with pathological reflexes including pyramidal signs and an abnormal pattern of movement and posture).
Visual MRI analysis: *WMR*= white matter reduction (MWMR=mild/moderate; SWMR=severe), *BG*=basal ganglia, *Thal*=thalamus, *MCA*=middle cerebral artery infarct, *HC*=hippocampus, periv=periventricular, f=frontal, cs=centrum semiovale, po=parieto-occipital.
NNS: neonatal seizures; *IQ*:Intelligence Quotient; *PIQ*: Performance IQ; *VIQ*: Verbal IQ

In the following sections, the findings from the VBM analyses are presented in more detail in the context of the categories for visual inspection of MR images (i.e. normal, abnormal, periventricular white matter abnormalities and, in particular, periventricular white matter reduction).

8.3.1 VBM-detected grey matter abnormalities in the group with normal MRI on visual inspection

In 9 of the 18 datasets judged as normal on visual inspection, VBM analysis did not detect any differences in grey matter density when compared with the VBM control group.

In 9 of the 18 datasets VBM analysis detected differences in grey matter density when compared to the VBM control group. Table 8.4 below summarises the location of VBM-detected grey matter abnormalities and whether an increase or decrease in grey matter was detected in the datasets from preterm children. In eight (TM, SSk, CS, MD, ASa, SDaW, BA, SF) of these nine datasets, the VBM detected grey matter abnormalities were focal (1-2 peaks). In these eight data sets the location was in the frontal lobe in two cases (in both cases increase of grey matter), in the temporal lobe in two cases (in both cases decrease of grey matter), in the thalami in two cases (increase of grey matter in both cases), in one case in the insular cortex (grey matter decrease) and in one case in the cingulate gyrus (increase of grey matter). In one of the nine datasets (JRu), more widespread VBM grey matter abnormalities were detected. In this dataset, grey matter increase in both thalami and the basal ganglia on the right was seen and grey matter decrease in both temporal lobes. Interestingly, in this child without epilepsy, EEG showed bilateral temporal sharp components (see appendix 6).

Table 8.4: VBM-detected differences in 9 out of the 18 preterm datasets that were normal on visual inspection. Location of differences in grey matter density in the individual datasets of preterm children compared to the VBM control group (n=16)

Location of VBM abnormalities	**Number of datasets with VBM abnormalities**	**Decrease/Increase in grey matter density compared to VBM control group**
Temporal lobe	2	↓
Frontal lobe	2	↑
Thalami (bilateral)	2	↑
Cingulate gyrus	1	↑
Insular gyrus	1	↓
Temporal lobe, thalami and basal ganglia	1	temporal lobe ↓ thalami and basal ganglia ↑

8.3.1.1 Interpretation of the VBM findings in the group with normal MRI on visual inspection

In all nine datasets without lesions on visual inspection of MRI, pre-processing of MR data gave good results for normalisation and segmentation. Therefore, the most likely explanation for the VBM findings is that they reflect true anatomical/biological differences between the preterm datasets and the VBM control group rather than differences introduced by a possibly suboptimal pre-processing process of datasets of abnormally shaped brains or brains with large lesions.

The decrease of grey matter density detected in the temporal lobe (BA, figure 8.21; SDaW, figure 8.16) and the insular cortex (TM, figure 8.8) can most likely be interpreted as regional volume loss /atrophy of grey matter in these regions.

The increase in grey matter detected in the frontal lobe (CS, figure 8.15; MD, figure 8.19), cingulate cortex (SSk, figure 8.9) and the thalami and/or basal ganglia (SF, figure 8.17; ASa, figure 8.13; JRu, figure 8.29) is more complicated to interpret. A possible explanation is that these abnormalities reflect abnormal distribution of grey matter within the thalami in

areas close to the primary white matter injury similarly to the pattern described in histo-pathological studies by Marin-Padilla (1997, 1999).

8.3.2 VBM-detected grey matter abnormalities in the group with abnormal MRI on visual inspection

In eight (30%; SD, MC, AT, LiWi, LWa, NSi, LH, RR) of the 27 datasets that were abnormal (in grey matter and/or white matter) on visual inspection, VBM did not detect any differences in grey matter density between the individual preterm datasets and the VBM control group. In three (LWa, NSi, LWa) of the eight children periventricular gliosis was seen on visual inspection, in three (MC, LH, SD) mild/moderate white matter reduction, in one bilaterally small hippocampi (AT), and in one child (RR) severe white matter reduction and abnormalities in the basal ganglia and thalami were seen on visual inspection.

In 19 (70%; for details see table 8.2, 8.3, and figures 8.8 to 8.35 for display of the statistical parametric maps on the individual subject's image) of the 27 datasets, VBM analysis detected differences in grey matter density when compared to the VBM control group. In 8 of these 19 datasets, VBM-detected abnormalities were focal (LB, BK, TC, SH, GO, BBe, DSk, NK), and in 11 more widespread (PD, WS, LR, JW, AM, ML, AU, EF, CO, DHay, LO).

In the following two sections, the VBM-detected abnormalities in these 19 datasets are described in more detail.

8.3.2.1 Focal VBM abnormalities

In 8 of the 19 datasets, focal grey matter abnormalities were detected by VBM. Table 8.5 below shows a summary of the findings.

The VBM-detected grey matter abnormalities were located in the cingulate cortex (in two datasets), in the temporal lobe (in two datasets), in the frontal lobe (in one dataset), in the cerebellum (in two datasets), in the parietal lobe (in one dataset), and the hippocampus (in one dataset).

In six of the eight datasets, visual inspection of the MR images had already identified grey matter abnormalities (see table 8.5 below; the dataset from DSk with the suspected Chiari I malformation is included here). In three of these 6 datasets (DSk, LB, NK) VBM analysis identified grey matter abnormalities that had been detected on visual inspection. In five (BBe, BK, GO, NK, LB) of the six datasets, VBM analysis failed to detect some (NK, LB) or all (BBe, BK, GO) grey matter abnormalities that had been seen on visual inspection of the images. These visible abnormalities were located in the hippocampi, thalami or basal ganglia. In all except two (LB, DSk) cases with visible grey matter abnormalities, VBM detected abnormalities in addition to those identified on visual analysis of MR images. In all four cases VBM abnormalities were seen in the same hemisphere as the grey matter abnormalities detected by visual inspection.

In the two datasets (SH, TC) in which only bilateral white matter reduction (and no grey matter abnormalities) had been seen on visual inspection of the images, VBM analysis detected cortical abnormalities. In one case (SH, figure 8.14), the VBM abnormalities were seen bilaterally (located close to the areas of white matter reduction) and in the other case (TC, figure 8.11) unilaterally.

In all datasets (except in one, SH) in which focal VBM abnormalities were detected, VBM analysis showed decreased grey matter density in the individual preterm datasets compared to the VBM control group.

8.3.2.1.1 Interpretation of the focal VBM findings

Pre-processing of all eight datasets, including the one dataset with severe white matter reduction and moderately enlarged ventricles, gave good results, so that the most likely explanation for the detected grey matter differences between the preterm datasets and the VBM control group are true anatomical/biological differences.

The decrease of grey matter density in the frontal (BBe, figure 8.18), temporal (BK, figure 8.12; NK, figure 8.23), parietal lobe (TC, figure 8.11) and the cingulate cortex (NK, SPM map of the findings in the cingulate cortex not shown) most likely represent focal grey matter loss/atrophy. In the two cases, in which a decrease in grey matter density in the cerebellum was detected (GO, figure 8.20; DSk, figure 8.22) the findings can be interpreted as cerebellar atrophy.

The increase of grey matter density seen bilaterally in the cingulate cortex in the dataset with bilateral severe white matter reduction (SH, figure 8.14) could be interpreted as abnormal distribution of grey matter in the sense of subtle acquired dysplasia following primary injury to the white matter. On inspection of the segmented images it appeared that the pre-processing procedure yielded good results, which support this interpretation. However, the VBM-detected abnormalities were located close to borders of white and grey matter, and since in this dataset there is also a likelihood that the enlarged ventricles may have led to a slight misclassification of white and grey tissue in the region of the borders between white and grey matter, it can not be ruled out that the detected differences may not reflect true anatomical/biological grey matter abnormalities.

In some datasets grey matter lesions that had been identified on visual inspection, were not identified by VBM analysis. In the majority of cases these were hippocampal abnormalities. The most likely cause for the VBM analysis not detecting abnormalities in the hippocampus is that the data in this study were smoothed to a 12 mm Gaussian Kernel; this makes the analysis most sensitive to detecting differences on this spatial scale, which makes the analysis relatively insensitive for detection of differences in structures as small

as the hippocampus. In two other datasets signal abnormalities on T1 and T2 weighted images in the caudate and/or thalamus had been seen but VBM analysis failed to detect differences between these datasets and the VBM control group. In these two cases too, the scale of smoothing might also have been on an inappropriate scale for detection of abnormalities in these structures. However, despite this, grey matter abnormalities in the thalami were detected by VBM in a number of other datasets in this study (see section 8.3.2.2 below), perhaps suggesting that the degree of thalamic abnormality may have played a role in VBM not detecting these abnormalities. Nevertheless, these findings suggest that in the two cases under discussion, the abnormalities that have been detected by VBM may err on the conservative side, since it was not possible (as described earlier in relation to single subject vs control group studies) to explore differences that may have been present on spatial scales smaller than the 12 mm smoothing limitation, and some smaller spatial scale abnormalities therefore may have remained undetected.

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Table 8.5: Summary of VBM-detected grey matter abnormalities in the datasets with focal VBM abnormalities in children with abnormal MRI on visual inspection (n=8)

Visual inspection of MR images *(subject's ID)*	**VBM: *Newly detected* gm abn. (not identified on visual inspection)**	**VBM: *Additional gm* abn. in those with gm abn. identified on visual inspection**	**VBM: *Confirmed* visible gm abn**	**VBM: Visible gm abn *missed***	**Increase/decrease in grey matter density compared to the VBM control group**
Hippocampi small bil *(BBe)*	Frontal lobe r	Yes	No	Yes (hippocampi)	↓
Caudate right *(BK)*	Temporal lobe r	Yes	No	Yes (caudate)	↓
Gliosis r>l, caudate r, thalamus l *(GO)*	Cerebellum l	Yes	No	Yes (thalamus, caudate)	↓
Gliosis l, hippocampi small bil, cortex temporo-parietal r (shunt) *(NK)*	Cingulate gyrus r	Yes	Yes (temporal lobe r)	Yes (hippocampi)	↓
MWMR bil *(TC)*	Parietal lobe r	n/a	n/a	n/a	↓
MWMR bil, Chiari I *(DSk)*	No	No	Yes (cerebellum l)	no	↓
MWMR bil, hippocampi small bil *(LB)*	No	No	Yes (hippocampus l)	Yes (hippocampus r)	↓
SWMR bil l>r, gliosis *(SH)*	Cingulate gyrus l and r	n/a	n/a	n/a	↑

R=right, l=left, bil=bilateral, abn=abnormalities, gm=grey matter, MWMR=mild/moderate white matter reduction, SWMR= severe white matter reduction; n/a=not applicable

8.3.2.2 Widespread VBM abnormalities

In 11 of the 19 datasets in which both visual inspection of the MR images and VBM analysis identified abnormalities, widespread differences in grey matter density between the preterm datasets and the VBM control group were detected. Table 8.6 below shows a summary of the findings. Figures 8.24 to 8.35 show statistical parametric maps superimposed on the individual subject's normalised image in order to help identify the anatomical location. In some cases, the SPMs were also superimposed on a mean image created from the normalised datasets of the controls. (Note: in some subjects, in whom multiple peaks were identified, only one fairly representative example of superimposed peaks was chosen for illustration.)

These VBM-detected grey matter abnormalities were located in the thalami (in nine datasets; 6/9 bilaterally), the parietal lobe (in eight datasets), temporal lobe (in seven datasets), frontal lobe (in five datasets), the cerebellum (in four datasets), cingulate cortex (in two datasets), basal ganglia (in two datasets), the occipital lobe (in one dataset), insula (in one dataset), and hippocampi (in one dataset).

In all 11 datasets periventricular white matter reduction was seen and in 6 (PD, AU, AM, ML, LO, JW) of the 11 datasets visual inspection of the MR images had identified grey matter abnormalities (see table 8.6 below; the dataset PD with bilaterally small cerebellum is included here). All visible grey matter abnormalities were confirmed by VBM, except in three cases (JW, LO, AU), in which the hippocampal abnormalities seen on visual inspection were not detected by VBM. In all six datasets with grey matter lesions identified on visual inspection, VBM detected additional grey matter abnormalities. The VBM-detected abnormalities were located unilaterally in the ipsilateral side to the abnormalities detected on visual inspection in one case (AM) and bilaterally in five cases (PD, AU, ML, LO, JW).

In 5 (DHay, WS, LR, CO, EF) of the 11 datasets only periventricular white matter reduction without additional grey matter abnormalities (n=2 mild/moderate and n=3 severe

white matter reduction) was seen on visual inspection of MRI (for a more detailed description of the findings in those with purely white matter lesions on visual inspection see section 8.3.2.3). In all five datasets, VBM detected differences in cortical grey matter between the preterm datasets and the VBM control group. The VBM-detected abnormalities were located in the parietal lobe (in five datasets), the temporal lobe (in three datasets), the frontal lobe (in three datasets), thalami (in two datasets), the occipital lobe (in one dataset), basal ganglia (in one dataset), hippocampus (in one dataset) and the cerebellum (in one dataset).

VBM abnormalities in the cortical grey matter and basal ganglia consisted of both increase and decrease in grey matter density compared to the VBM control group. VBM-detected abnormalities in the cerebellum showed decrease of grey matter density. Except in one case (CO), abnormalities in the thalami consisted of increase in grey matter density.

8.3.2.2.1 Interpretation of the widespread VBM findings

In some of these 11 datasets, large lesions (dilated ventricles and/or cortical defects) were present. As described above in the methods section (8.2.1), attempts have been made to account for this, for example, masking of the lesions for optimisation of the normalisation procedure. These datasets were assessed visually for successful or failed normalisation and segmentation and data with a poor pre-processing result were excluded (for details see appendix 7). However, even in the included datasets there is still a possibility that some of the VBM-detected grey matter abnormalities do not reflect true anatomical/biological differences between the preterm datasets and the VBM control group, but are caused by misclassification of tissue during the segmentation process. This has to be kept in mind when interpreting the VBM findings in these datasets.

In all datasets except one (CO), both decrease and increase of grey matter density was detected by VBM. For some of these findings the interpretation is not straightforward. The most likely interpretation is that in these cases, in addition to the visible lesions, subtle

widespread brain abnormalities, with both areas of atrophy and areas with aberrant distribution of grey matter (in the sense of changes in the cytoarchitecture of grey matter) are present.

In the majority of the cases, decrease in grey matter density was seen in similar regions to those with focal VBM-detected grey matter abnormalities and no large visible lesions, i.e. the frontal lobe, parietal lobe, temporal lobe, insular and cingulate gyrus, the thalamus and the cerebellum. This might indicate that in these 11 datasets the VBM-detected decrease of grey matter is in fact a true anatomical/biological finding rather than an artifactual finding. On the other hand, as already mentioned above, in some of these datasets some of the findings may in fact likely to be caused by misclassification of tissue during segmentation (in particular in the datasets of JW, AU, LR, ML, LO). However, in all datasets with visible cortical grey matter lesions (AM, ML, LO, JW), VBM analysis detected these abnormalities correctly as a decrease in grey matter density (see table 8.6 below). This further supports the interpretation that the VBM-detected decrease in cortical grey matter reflects true anatomical/biological differences between the preterm children and the VBM control group, i.e. subtle volume loss of grey matter within a region or more global atrophy of a structure (e.g. the cerebellum). Similarly to the datasets with focal VBM abnormalities (section 8.3.2.1), the abnormalities that were seen on visual inspection but missed by VBM, were located in the hippocampi (JW, LO, AU) and the choice of the size of the smoothing kernel is the most likely reason for not detecting these abnormalities.

Increase in grey matter density in the cortical grey matter is complicated to interpret, as in the datasets with focal VBM abnormalities. In some cases with mild/moderate or severe periventricular white matter reduction, it might be interpreted as abnormal grey matter distribution (in the sense of acquired focal dysplasia) following a primary insult to the periventricular white matter. In some of those with bilateral periventricular white matter reduction and additional visible cortical grey matter lesions such as infarcts or lesions caused by a shunt (e.g. AM, LO, ML), the finding that VBM detected areas of increased grey matter density in the contra lateral side to the side affected by the shunt or infarct can be viewed as supporting this interpretation. In the case of JW (bilateral schizencephaly and

adjacent polymicrogyria and severe periventricular white matter reduction) VBM detected decreased grey matter density compared to the VBM control group in the area of the clefts and the atrophied temporal lobes, and increased grey matter density in the region of the polymicrogyria. These findings, too, indicate that even in abnormally shaped brains VBM can, to a certain extent, correctly identify anatomical abnormalities in grey matter between a lesioned brain and a healthy brain.

Increase of grey matter density in the thalami in the preterm datasets compared to the VBM control group was a very frequent finding. The possible ways of interpreting these findings are discussed in detail further below.

In general, in this subgroup with abnormal MRI on visual inspection and widespread VBM-detected abnormalities, cautious interpretation of the VBM findings is necessary since pre-processing of the MR data in such datasets may result in misclassification of tissue in some regions. In such datasets, although VBM correctly identifies differences in grey matter between a subject's dataset and the datasets from the healthy control group, in some brain regions VBM findings might not reflect true anatomical/biological differences but rather artefacts introduced by the pre-processing of the data.

Table 8.6: Summary of VBM-detected grey matter abnormalities in the datasets with widespread (>=3) VBM abnormalities in children with abnormal MRI on visual inspection (n=11)

Visual inspection of MR images *(subject's ID)*	**VBM: *Newly detected* gm abn. (not identified on visual inspection)**	**VBM: *Additional* gm abn. in those with gm abn. detected on visual inspection**	**VBM: *Confirmed* visible gm abn.**	**VBM: Visible gm abn *missed***	**Increase/decrease in grey matter density compared to the VBM control group**
MWMR bil *(DHay)*	Frontal lobe r Parietal lobe l Thalami bil	n/a	n/a	n/a	Frontal, parietal lobe ↓ Thalami ↑
MWMR bil Gliosis *(WS)*	Occipital lobe r Frontal lobe r Parietal lobe r Cerebellum l Thalamus r	n/a	n/a	n/a	Frontal, parietal lobe, cerebellum ↓ Occipital lobe, thalamus ↑
MWMR bil Gliosis Cerebellum small bil *(PD)*	Temporal lobe r Thalami bil	n/a	Yes (Cerebellum)	n/a	Temporal lobe, cerebellum ↓ Thalami ↑
SWMR bil *(LR)*	Parietal lobe r Temporal lobe r Hippocampus r Basal ganglia r Thalami bil	n/a	n/a	n/a	Parietal, temporal lobe, hippocampus, basal ganglia ↓ Thalami ↑
SWMR bil *(CO)*	Parietal lobe r Temporal lobe r Thalamus r	n/a	n/a	n/a	Parietal, temporal lobe, thalamus ↓
SWMR bil Gliosis *(EF)*	Frontal lobe l Temporal lobe r Parietal lobe bil Cerebellum l	n/a	n/a	n/a	Parietal lobe, cerebellum ↓ Frontal, temporal lobe ↑
SWMR bil Hippocampus r	Temporal lobe r Fronal lobe l	Yes	no	Yes (hippocampus)	Temporal, frontal lobe, cingulate ↓ Thalami ↑

(AU)	Cingulate gyrus l Thalami bil				
SWMR bil Gliosis Cortex temporal-parietal r *(AM)*	Cingulate r	Yes	Yes (Temporal, parietal lobe r)	no	Temporal-parietal ↓ Parietal lobe, cingulate ↑
SWMR bil Gliosis Cortex (MCA) l Hippocampus l Cerebellum, pons *(ML)*	Thalami bil	Yes	Yes (Temporal, parietal lobe l, Cerebellum Hippocampus/amygdala l)	no	Temporal, parietal lobe, amygdala/hippocampus, cerebellum ↓ Temporal, parietal lobe, Thalami ↑
SWMR bil Cortex parietal l (shunt) Hippocampus l Cerebellum *(LO)*	Frontal lobe bil Temporal lobe r Parietal lobe r Frontal lobe bil Thalamus r Basal ganglia l	Yes	Yes (Parietal, temporal lobe l Cerebellum l)	Yes (hippocampus l)	Temporal, parietal lobe, cerebellum ↓ Frontal lobe, basal ganglia, thalamus ↑
SWMR bil Schizencephaly bil Polymicrogyria bil Hippocampus bil *(JW)*	Parietal lobe bil Insula l Temporal lobe bil Thalami bil Cerebellum l	Yes	Yes (Frontal, parietal lobe bil)	Yes (hippocampi)	Temporal, frontal, parietal lobe, cerebellum, insula ↓ Frontal, parietal lobe, thalami ↑

R=right, l=left, bil=bilateral, abn=abnormalities, gm=grey matter, MWMR=mild/moderate white matter reduction, SWMR= severe white matter reduction; n/a=not applicable

8.3.2.3 VBM-detected grey matter abnormalities in the subgroup with periventricular white matter reduction

One of the main hypotheses of this thesis is that in preterm children, epilepsy and/or cognitive impairment are associated with subtle grey matter abnormalities that are additional to the white matter abnormalities typically seen in preterm children. Thus, it is of particular interest to examine the group with periventricular white matter lesions in more detail with regard to the detection of subtle grey matter abnormalities by VBM analysis in those without visible grey matter lesions. The VBM findings in these datasets have been described in previous sections but specific emphasis is placed on children with periventricular white matter reduction who did not have additional visible grey matter abnormalities (e.g. MCA infarcts, malformations, or cortical lesions associated with shunt insertion).

Periventricular white matter abnormalities were seen in 32 MRI datasets on visual inspection (see chapter 7; gliosis only without white matter reduction n=5, white matter reduction with or without gliosis n=27). VBM analysis was performed in all 5 datasets with gliosis only and in 19 of the datasets with white matter reduction. In 2 of the 5 datasets with gliosis only, and in 7 of the 19 datasets with white matter reduction, grey matter abnormalities were already seen on visual inspection of the images. These datasets will not be further considered in this section. In 14 datasets white matter abnormalities only and no grey matter abnormalities were seen on visual inspection (n=3 with gliosis only, n=11 with periventricular white matter reduction with or without gliosis. Note: the two datasets, PD, DSk, with small cerebellum on visual inspection are included here). In the 11 datasets with white matter reduction and no grey matter abnormalities on visual inspections, the degree of white matter reduction was judged as mild/moderate in seven and as severe in four cases.

In the 3 datasets with gliosis only, no differences in grey matter density between the individual preterm datasets and the VBM control group were detected by VBM. In contrast, in 9 (TC, SH, DSk, WS, LR, EF, CO, DHay, PD) of the 11 datasets with periventricular

white matter reduction (with or without gliosis), VBM detected differences in grey matter density.

Figure 8.7 below shows the distribution of VBM-detected grey matter abnormalities in the group with periventricular white matter abnormalities and no grey matter abnormalities identified on visual inspection of MR images. Although the numbers in this subgroup are small, statistical testing indicated that the degree of white matter reduction is correlated with the number of VBM-detected grey matter abnormalities, i.e. in those with more severe degree of white matter reduction, more widespread subtle grey matter damage was present (Spearman's ρ=0.7, p=0.009).

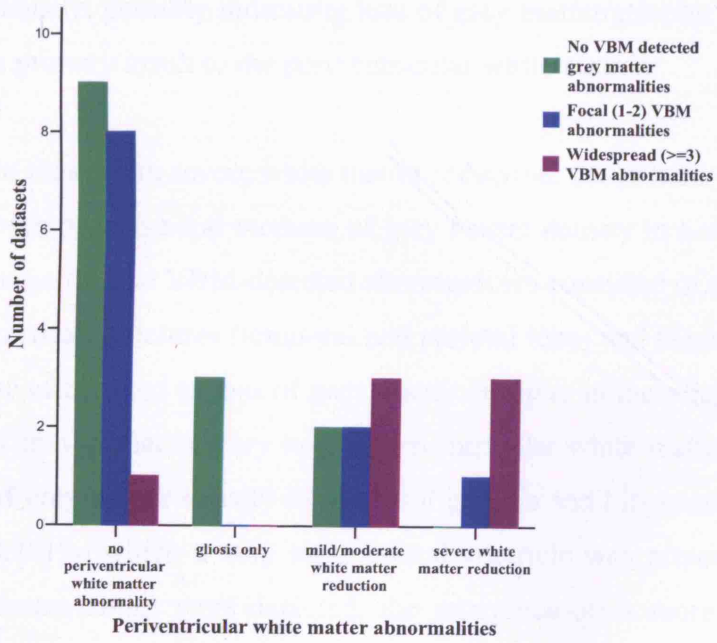


Figure 8.7: Distribution of VBM abnormalities in the 14 datasets (displayed in the three clustered bars to the right on the X-axis) in which periventricular white matter abnormalities were seen without grey matter abnormalities on visual inspection of MR images. Gliosis only n=3, mild/moderate white matter reduction with or without gliosis n=7, severe white matter reduction n=4. In 5 datasets with periventricular white matter abnormalities (n=3 with gliosis only, n=2 with mild/moderate white matter reduction) VBM did not detect any abnormalities.

8.3.2.3.1 Interpretation of the VBM findings in the group with periventricular white matter abnormalities and no visible grey matter lesions

The findings in this subgroup have in part been discussed in section 8.3.2.2.1 above. In this section, they will be discussed specifically in the context of the periventricular white matter lesions identified on visual inspection.

The VBM-detected grey matter abnormalities in these datasets consisted of both increase and decrease in density of grey matter compared to the VBM control group.

In those with mild/moderate white matter reduction, abnormalities mainly in the frontal lobes, temporal lobes, parietal lobes and the cerebellum showed decrease in grey matter density, possibly indicating loss of grey matter/atrophy in these regions in association with a primary insult to the periventricular white matter.

In those with severe white matter reduction, the pattern was less uniform. In some datasets, both decrease and increase of grey matter density in cortical structures was detected. In the majority, the VBM-detected abnormalities consisted of decreased grey matter density in the cortical structures (temporal and parietal lobe) and the cerebellum, and most likely this can be interpreted as loss of grey matter /atrophy in these regions subsequently to or associated with a primary injury to the periventricular white matter. Regarding the detected decrease of grey matter density in the basal ganglia and hippocampus in one dataset (LR, see figure 8.28) in which a very large lateral ventricle was present on the side on which the VBM abnormalities were detected, the interpretation is more complicated and the findings are probably at least in part caused by misclassification of tissue rather than true anatomical differences. Similarly, in the dataset of CO (see figure 8.35), the very large ventricles might have led to misclassification of tissue in the temporal lobe and the region of the thalami. In the dataset (EF, see figure 8.34) in which both increase and decrease of grey matter in the cortex was seen, the decrease in the parietal lobe and the cerebellum most likely reflects subtle grey matter loss atrophy in these cortical region and/or, in the case of the cerebellum, atrophy of the structure. The increase in the frontal and temporal lobe might, in this case,

rather be due to abnormal distribution of grey matter than to artefacts introduced by the data processing, since misclassification of tissue in these regions seems less likely than in regions closer to the lateral ventricles.

In all of the datasets, except one (EF), VBM analysis detected abnormalities in the thalami. In the majority of the cases these abnormalities consisted of an increase in grey matter density. The interpretation of these VBM findings in the thalami is discussed in the next section.

As already discussed in the sections above, it is difficult to judge whether these findings represent true anatomical/biological differences between the preterms and the VBM control group or whether some of the findings are caused by the pre-processing procedure in brains with large lesions. In general, in such datasets, caution is required when interpreting the results. However, overall the findings indicate that in those with periventricular white matter reduction (even in those without visible grey matter lesions), widespread grey matter abnormalities are present that are detected by VBM. In addition, the findings indicate that the severity of white matter reduction is associated with the number of the detected subtle grey matter abnormalities.

8.3.2.4 VBM-detected abnormalities in the thalami and basal ganglia

Subtle abnormalities in basal ganglia and mainly in the thalami that may not be identifiable on visual inspection of MR images have been described in preterm infants previously, mainly in the context of white matter abnormalities (e.g. Srinivasan et al, 2007; Boardman et al, 2006; see chapter 7), supporting the view that white matter injury in preterms is not an isolated phenomenon but associated with injury to other structures. In this section, VBM abnormalities in the thalami and basal ganglia detected in the current study are explored in detail and it is investigated how these VBM-detected abnormalities are associated with periventricular white matter lesions.

In 12 (43%; for details see tables 8.2 and 8.3) of the 28 datasets in which VBM identified differences in grey matter density between the MR data of the preterm children and the VBM control group, abnormalities in the thalami were detected. These abnormalities were bilateral in 8 of the 12 datasets. Abnormalities in the thalami had not been identified in any of these datasets on visual inspection of the MR images.

In three cases (SF, ASa, JRu) MRI images had been judged as normal on visual inspection. In nine cases (PD, WS, JW, ML, AU, DHay, CO, LO, LR) MRI was abnormal on visual inspection and in all nine datasets periventricular white matter reduction was present. In five of these nine datasets only white matter lesions and no grey matter lesions had been detected on visual inspection. In four datasets one or more grey matter abnormalities had been seen (cortical and/or hippocampal abnormalities).

In all cases except two (SF, ASa; both had normal MRI on visual inspection and the thalamus abnormalities were the only VBM detected differences when compared to the VBM controls), the VBM-detected abnormalities in the thalami were associated with additional, more widespread, VBM abnormalities. In three cases (LR, JRu, LO) abnormalities in the basal ganglia were detected as well.

In all cases but two (CO, decrease in grey matter of the thalamus; LR, decrease of grey matter density in basal ganglia) VBM analysis showed increased grey matter density in the thalami and basal ganglia in the preterm datasets when compared to the VBM control group.

8.3.2.4.1 Interpretation of the VBM findings in thalami and basal ganglia

The increase of grey matter density in the thalami in the preterm datasets that were normal on visual inspection most likely reflects a true anatomical/biological difference to the VBM control group. It is less likely that in these datasets the differences were caused by misclassification of tissue since no large abnormalities in the periventricular white matter

were present that might result in sub-optimal segmentation results. Thus, the findings in these three datasets might be interpreted as abnormal distribution of grey matter in the thalami subsequently to subtle early brain injury. In the datasets in which obvious periventricular white matter lesions are present (in particular in those with severe white matter reduction) the VBM-detected increase in grey matter density might not reflect true anatomical/biological grey matter abnormalities in the thalami/basal ganglia but may rather be caused by artefacts, i.e. in regions where injured and subsequently "atrophic" white matter is close to grey matter structures, VBM might erroneously detect in these areas increased grey matter density. However, on the other hand, in more extensive brain injury in preterms, involvement of the thalami has been described thus supporting the interpretation that these findings are true anatomical/biological differences between the preterms and the VBM control group.

In the two datasets in which a decrease of grey matter was detected in the thalamus and basal ganglia respectively, the most likely interpretation is atrophy of these regions in the context of widespread lesions. In both cases, severe white matter reduction with subsequent ventricular dilatation was seen on visual inspection, indicating severe perinatal brain injury.

8.3.3 Distribution of VBM-detected grey matter abnormalities between the group with and the group without epilepsy and in relation to cognitive function

This is described and discussed in chapters 9 and 10.

8.3.4 Summary of the main results

VBM detected subtle grey matter abnormalities in 62% of the datasets that were included in the analysis. The presence or absence of VBM-detected abnormalities was not significantly associated with the presence or absence of abnormalities on visual inspection of MRI images. However, the number of VBM-detected abnormalities was significantly associated with the presence or absence of abnormalities detected on visual inspection of the MR images.

In 50% of those with normal MRI on visual inspection, VBM did detect differences in grey matter density between the preterm datasets and the VBM control group. These abnormalities were focal in all but one case and consisted of a decrease in grey matter density in the majority of the cases.

In the majority (70%) of those with abnormal MRI on visual inspection, VBM detected grey matter abnormalities. The frequency of focal (n=8) and widespread (n=11) VBM abnormalities in those with abnormal MRI was not very different. There was a significant association between the presence of periventricular white matter abnormalities identified on visual inspection and the number of VBM-detected grey matter abnormalities. Furthermore, the degree of white matter reduction was significantly associated with the number of VBM-detected abnormalities in grey matter.

Abnormalities were seen most frequently in the temporal lobe, parietal lobe, thalamus, frontal lobe and the cerebellum. Abnormalities were found less frequently in the cingulate cortex, insular cortex, basal ganglia and hippocampi.

In those with focal abnormalities, mainly a decrease in grey matter density was detected and in those with widespread VBM abnormalities both decreases and increases in grey matter density was detected. Abnormalities in the thalami in the majority consisted of an increase in grey matter density. Abnormalities in supratentorial cortical regions mainly

showed a decrease in grey matter density and less frequently increase of grey matter density. Abnormalities in the cerebellum consisted of a decrease in grey matter density.

It has to be kept in mind that in a number of the datasets with large lesions, some of the VBM findings might not reflect true anatomical/biological grey matter differences between the preterms and the VBM control group and thus VBM findings in this subset have to be interpreted with caution.

Figures 8.8 – 8.35: Statistical parametric maps superimposed on the individual subject's normalised image. The Z scores obtained from the statistical testing are indicated by the colour according to the scale as shown in figure 8.3.In some cases the SPMs were also superimposed on a mean image created from the normalised datasets of the controls. This additional method of display of results was chosen for subjects in whom there was evidence that controls might have more grey matter and the abnormalities detected exceeds the patient's data extent (i.e. if the peaks appeared to be outside the patient's brain). The maps are displayed at p=0.0001 uncorrected. Images are displayed in neurological convention (left=left, right=right), and the crosshairs show the location of the maximal peak. In some subjects, in whom multiple peaks were identified, only one fairly representative example of superimposed peaks was chosen for illustration.

a) Datasets in which VBM grey matter analysis detected focal (1-2 peaks) abnormalities.

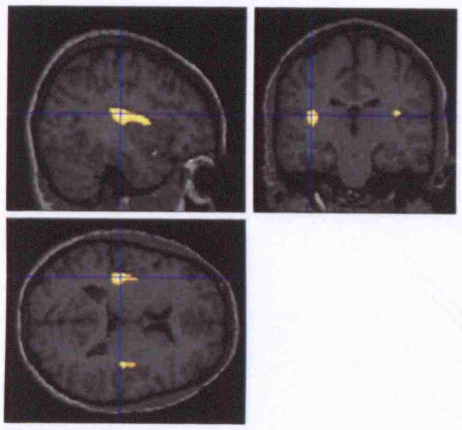


Figure 8.8: TM, decreased grey matter in left insular gyrus.

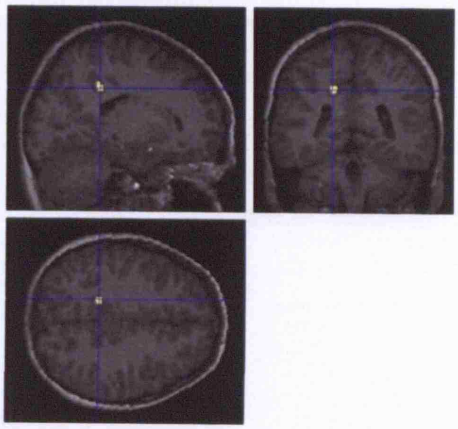


Figure 8.9: SSk, increased grey matter in left cingulate gyrus.

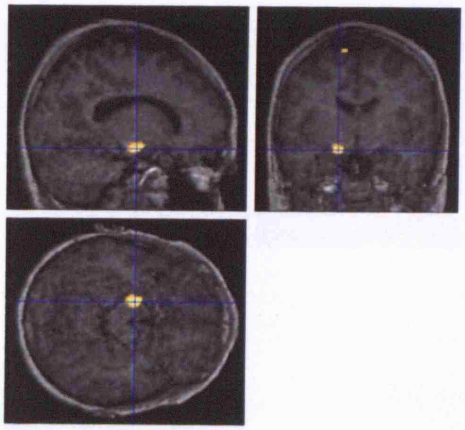


Figure 8.10: LB, decreased grey matter in left hippocampus.

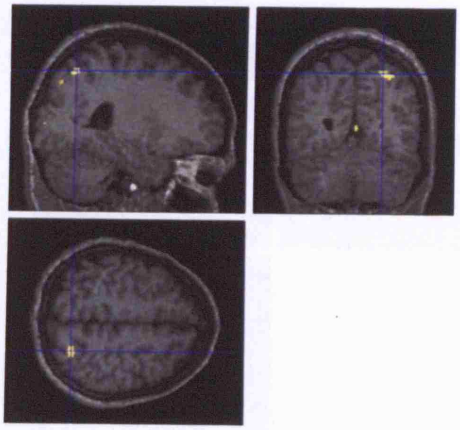


Figure 8.11: TC, decreased grey matter in right parietal lobe.

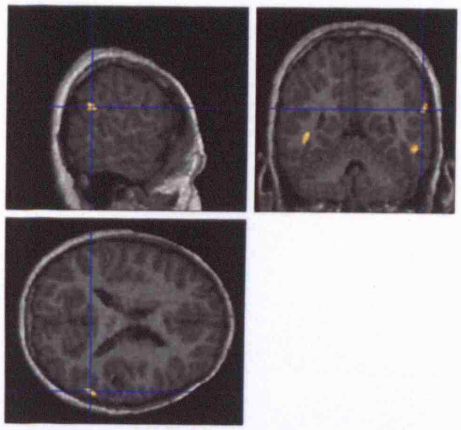


Figure 8.12 BK, decreased grey matter right temporal lobe.

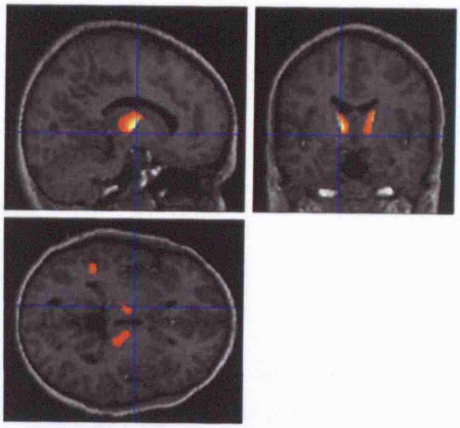


Figure 8.13: ASa, increased grey matter thalami bilaterally.

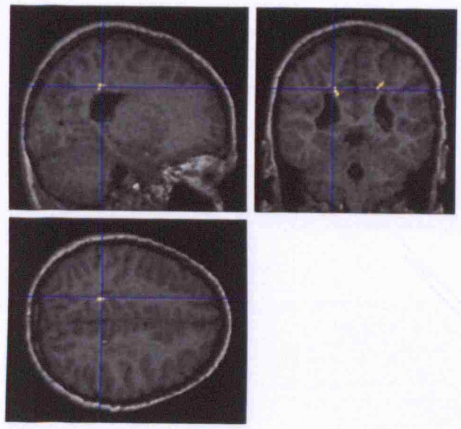


Figure 8.14: SH, increased grey matter cingulate gyrus bilaterally.

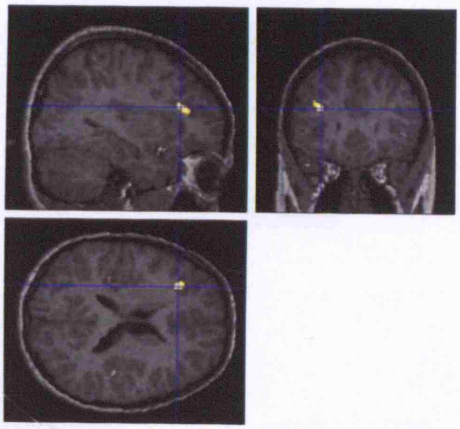


Figure 8.15: CS, increased grey matter left frontal lobe.

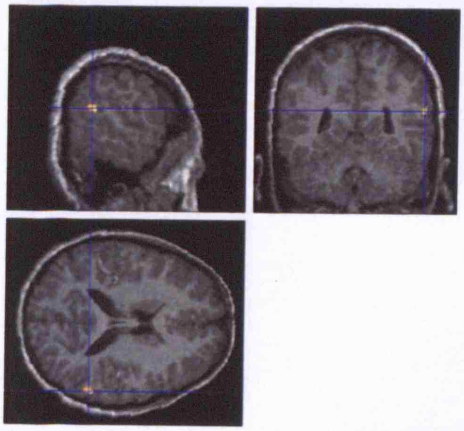


Figure 8.16: SDaW, decreased grey matter right temporal lobe.

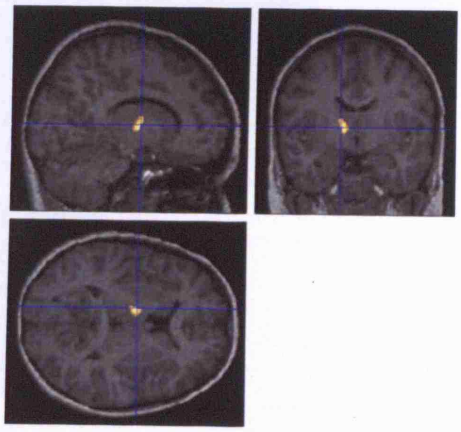


Figure 8.17: SF, increased grey matter left thalamus.

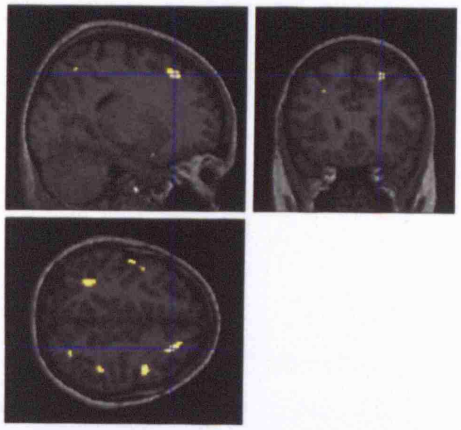


Figure 8.18: BBe, decreased grey matter right frontal lobe.

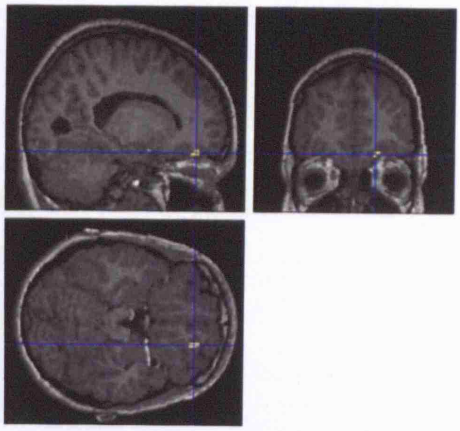


Figure 8.19: MD, increased grey matter right frontal lobe.

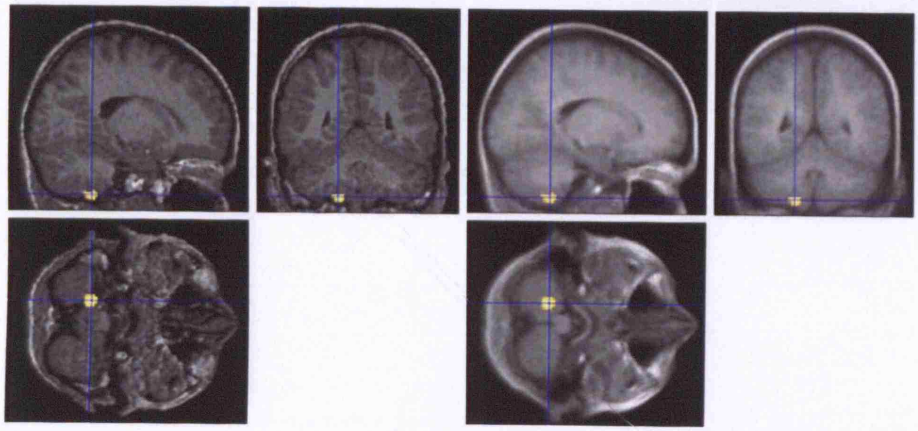


Figure 8.20 a and figure 8.20 b: GO, decreased grey matter left cerebellum. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

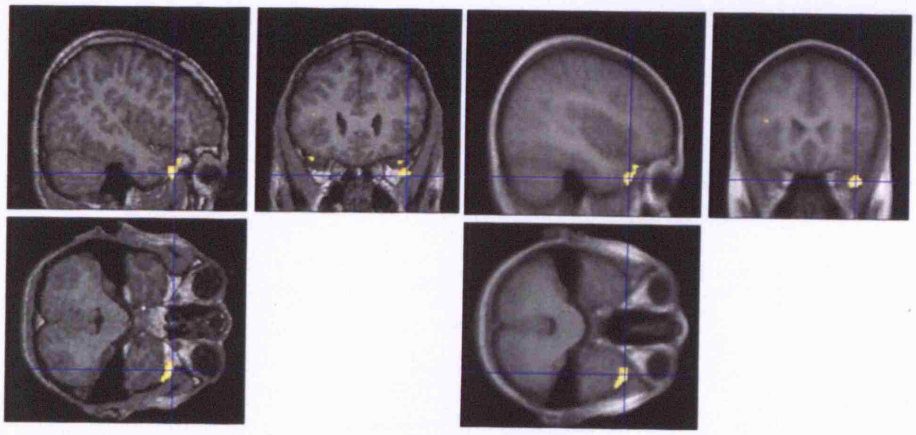


Figure 8.21 a and figure 8.21 b: BA, decreased grey matter in right temporal lobe. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

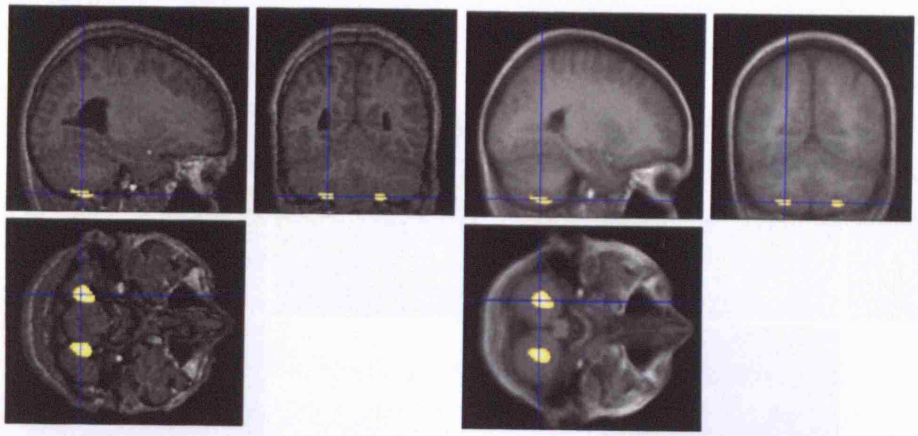


Figure 8.22 a and figure 8.22 b: DSk, decreased grey matter in the left cerebellum. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

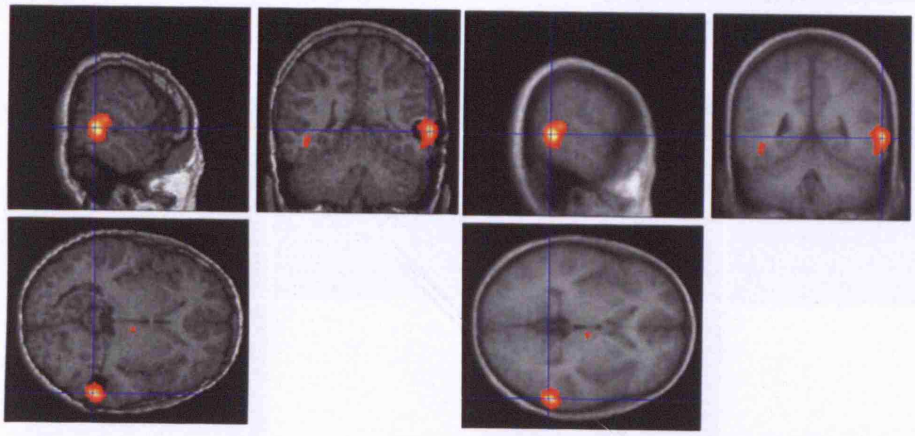


Figure 8.23 a and figure 8.23 b: NK, decreased grey matter right temporal lobe. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

b) Datasets in which VBM grey matter analysis detected widespread (>=3 peaks) abnormalities

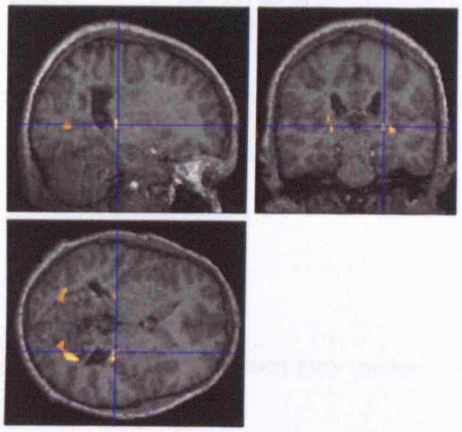


Figure 8.24; WS, increased grey matter right thalamus.

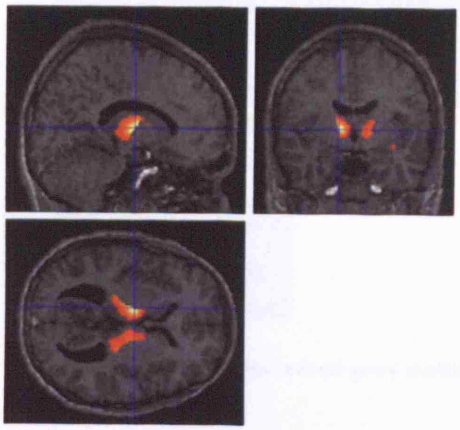


Figure 8.25: DHay, increased grey matter. left thalamus.

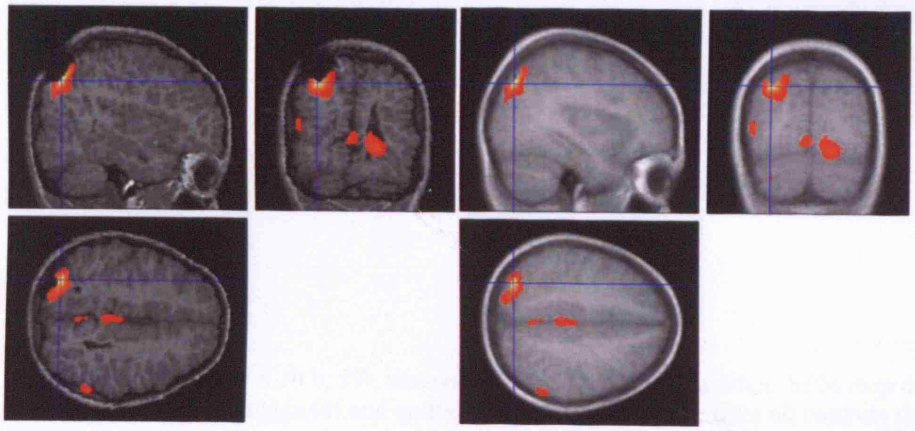


Figure 8.26 a and figure 8.26 b: LO, decreased grey matter left parietal lobe. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

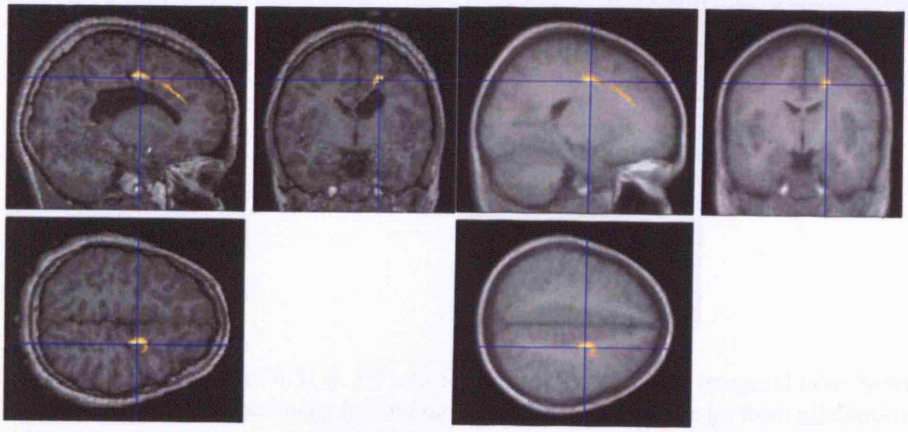


Figure 8.27 a and figure 8.27 b: AM, decreased grey matter right temporo-parietal region. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

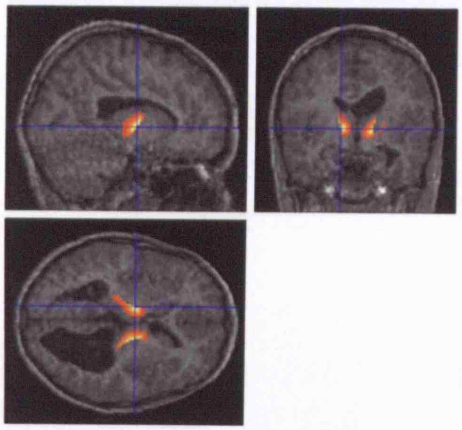


Figure 8.28: LR, increased grey matter left thalamus.

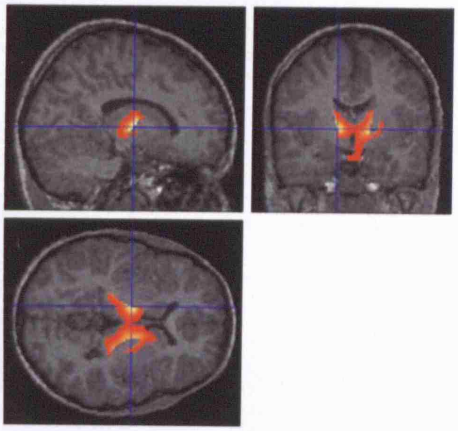


Figure 8.29: JRu, increased grey matter left thalamus.

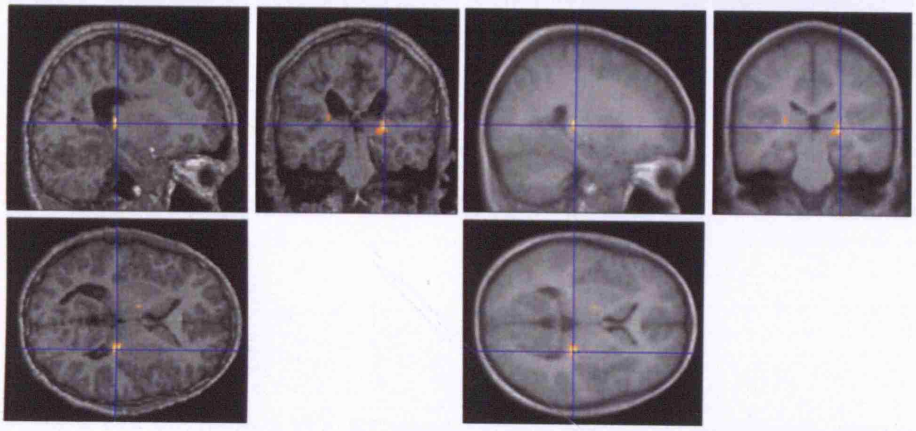


Figure 8.30 and Figure 8.30 b: PD, increased grey matter right thalamus. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

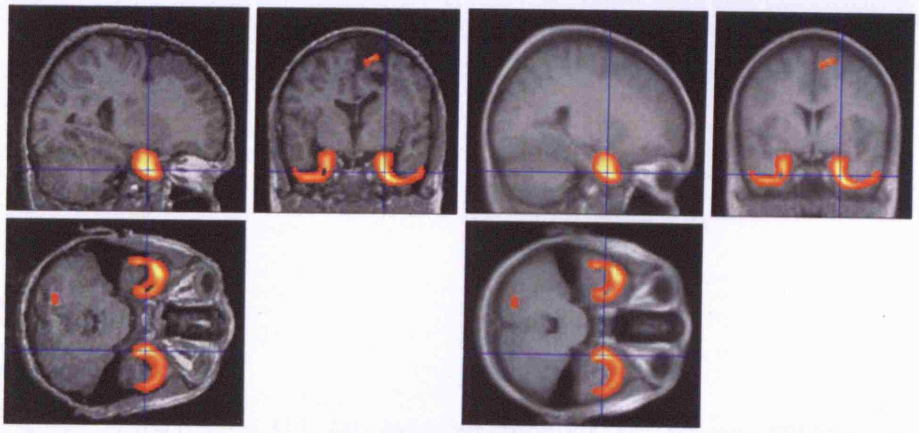


Figure 8.31 a and Figure 8.31 b: JW, decreased grey matter right temporal lobe. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

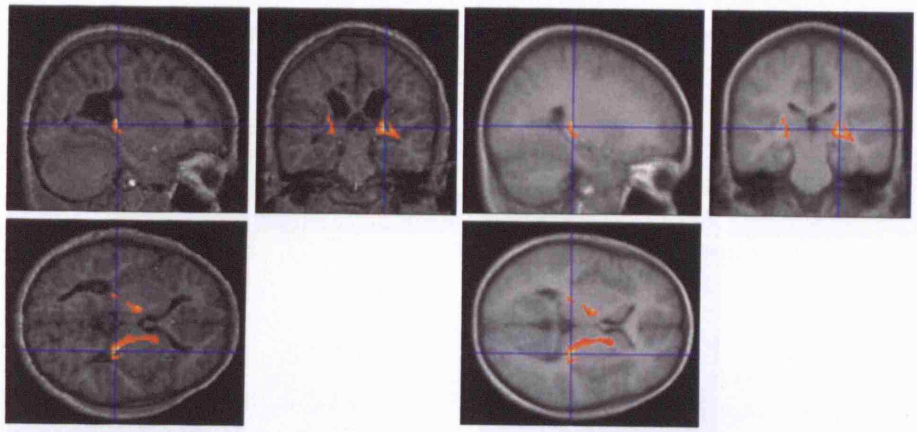


Figure 8.32 a and figure 8.32 b: AU, increased grey matter right thalamus. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

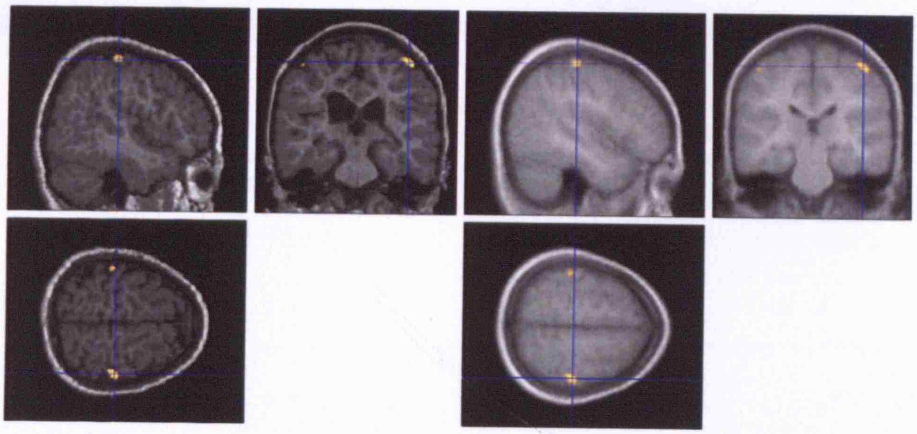


Figure 8.33 a and figure 8.33 b: EF, decreased grey matter right parietal lobe. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

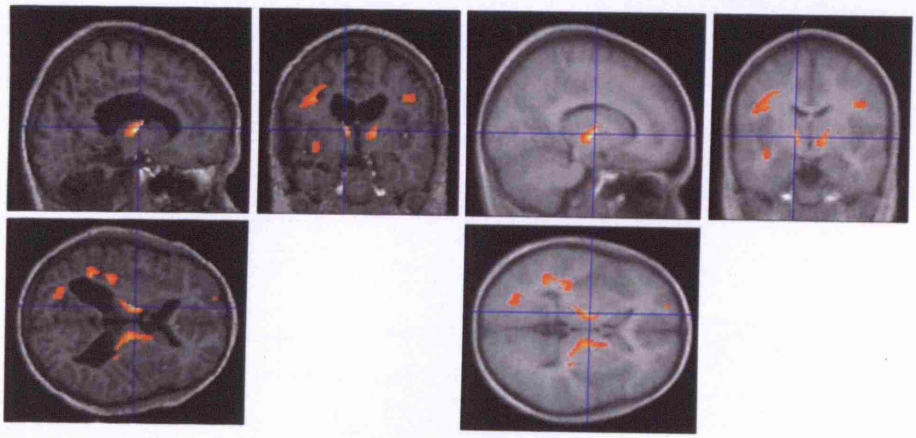


Figure 8.34 and figure 8.34 b: ML, increased grey matter left thalamus. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

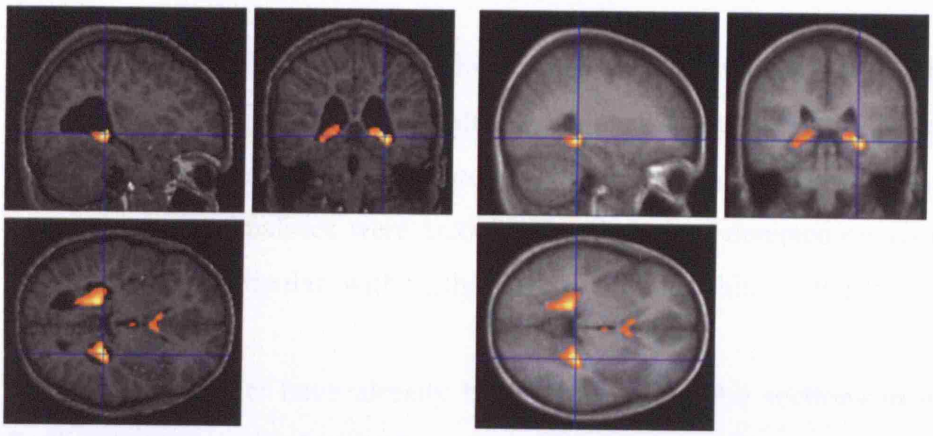


Figure 8.35 a and figure 8.35 b: CO, decreased grey matter in region of right temporal lobe. SPM map displayed on the subject´s normalised image (a) and on the mean smoothed image from all controls (b).

8.4 Discussion

The main questions addressed in this chapter were first, whether VBM analysis would identify any grey matter abnormalities in the MR data sets of the preterm children included in the analysis (n=45). Second, it was investigated how these VBM-detected grey matter abnormalities were associated with lesions detected on visual inspection of MR images, in particular, with visible periventricular white matter lesions.

Some of the results have already been discussed in the sections in which the VBM findings were described and reference to these sections is made where appropriate.

As outlined in section 8.2 above, VBM was initially developed to examine differences in brain structure between groups of adult subjects. There are a number of methodological issues that need to be considered when VBM is used for analysis of individual MRI datasets obtained from children, some of which have visible brain lesions. In this study, attempts have been made to optimise the pre-processing procedure and adapt it to the data available for analysis. This has been described in detail in section 8.2.1 and a more general discussion of some methodological issues is presented at the end of this discussion. It is important to keep in mind that in this study the interpretation of the results obtained from VBM analysis might be confounded by some of these methodological limitations.

Of the 51 available 3D MPRAGE datasets, 45 were entered in the VBM analysis. Six datasets had to be excluded since one or more pre-processing steps failed. In all six datasets large periventricular white matter lesions were present and five of the six children whose datasets were excluded from analysis had epilepsy, cognitive impairment and, except one, all had abnormal neurological signs. The exclusion of the data from these children might introduce a bias when investigating the associations between the VBM findings and outcome (presented in subsequent chapters).

In 62% of the preterm datasets subtle grey matter abnormalities were detected by VBM analysis. In 32% of the datasets in which VBM grey matter abnormalities were detected, visual inspection of MRI had been normal and in 68% abnormalities had been detected on visual inspection. Interestingly, statistical testing did not show a significant

association between the presence/absence of VBM abnormalities and normal/abnormal MRI on visual inspection (see section 8.4.4.2 for discussion). However, a significant association was found between the number (categorised as none or focal (1-2 peaks), widespread (>=3 peaks)) of VBM-detected abnormalities and normal or abnormal MRI on visual inspection, indicating that in those with visible lesions more additional widespread grey matter damage is present. Further testing indicated that this relationship between lesions identified on visual inspection and subtle grey matter abnormalities detected by VBM is mainly explained by the presence of periventricular white matter lesions, and in particular, the severity of white matter reduction, and not by the presence of visible grey matter lesions. (Note: in some of the analyses the numbers were small and this has to be kept in mind when interpreting the results obtained from statistical testing.)

8.4.1 VBM-detected increase and decrease in grey matter density

In this study, VBM analysis detected both decreases and increases in grey matter density compared to the healthy term born controls. A decrease was mainly found in the datasets in which focal VBM abnormalities were present and in those datasets that had no or mild/moderate lesions on visual inspection of the images. As already discussed in section 8.3.2.1.1, this most likely reflects regional atrophy in the affected regions and these findings are consistent with previous studies in prematurely born children and adolescents/young adults who have survived without large brain lesions. For example, Isaacs et al (2001, 2003, 2004) in a series of studies in preterm children and adolescents used VBM to investigate brain structure and associations with overall cognitive function (Isaacs et al, 2004) and/or specific cognitive deficits (Isaacs et al, 2001, 2003). In these studies VBM detected focal brain abnormalities consisting of decrease in grey matter density in the temporal lobe (associated with visuo-spatial processing deficit; Isaacs et al, 2003), the parietal lobe (associated with calculation difficulties; Isaacs et al, 2001), both the parietal and temporal lobe (associated with absolute IQ scores; Isaacs et al, 2004) and abnormalities in the frontal, temporal, and occipital lobes (associated with decline over time in VIQ and PIQ respectively; Isaacs et al, 2004). The majority of the children in these studies had normal MRI on visual inspection and only few had

minor/moderate brain abnormalities on visual inspection of the images including a small corpus callosum, delay of myelination, mild "PVL" and/or small hippocampi.

Allin et al (2004), in a study in adults (mean age 23 years) born with very low birth weight (birth weight < 1500 g, gestational age 26-37 weeks) investigated brain structure using computational morphometry with an automated tissue segmentation algorithm. They found widespread changes in grey and white matter consisting of decrease and increase in grey matter density in the brains of the subjects born preterm when compared to tem born controls. Similarly to the regions in which VBM detected abnormalities in the current study, in this study, the affected regions were located in the medial temporal, frontal, parietal lobe, the cingulate cortex, thalami, basal ganglia and parahippocampal structures. Some of the subjects in the study by Allin et al (2004) had dilated lateral ventricles and there was a significant association between increased volume of the lateral ventricles and decreased grey matter density in subcortical nuclei and limbic cortical structures.

In the current study, abnormalities in the cerebellum consisted of decrease in grey matter density and this was frequently associated with supratentorial abnormalities in both visual inspection of the MR images and grey matter abnormalities detected by VBM. These findings can be regarded as consistent with the findings of previous studies that found that smaller cerebellar volume in preterm children measured at term age (e.g. Limeropoulos et al, 2005; Srinivasan et al, 2006) or later in childhood (Allin et al, 2005) was associated with supratentorial white matter lesions and/or reduced white matter volume.

Increase in grey matter density was mainly detected in the thalami. In most cases, these abnormalities were bilateral and associated with periventricular white matter reduction. In all but two cases, the VBM-detected abnormalities in the thalami were associated with other, more widespread VBM abnormalities. Possible interpretations of these findings have been discussed in section 8.3.2.4.1. Abnormalities in the thalami and basal ganglia detected by quantitative MR analysis methods have been described in previous studies in preterm populations and in most studies these abnormalities were associated with white matter damage (e.g. Boardman et al, 2006; Srinivasan et al, 2007) However, in contrast to the current study, thalamus and basal ganglia abnormalities in

most studies consist of volume reduction or decrease in grey matter density (e.g. Allin et al, 2004) and not in increase of grey matter density. As already discussed in section 8.3.2.4.1, it might be possible that these VBM findings are not caused by true anatomical/biological differences in grey matter between the preterms and the term controls but by misclassification of tissue in areas close to injured and atrophic white matter. However, on the other hand, the finding of abnormal grey matter density (either increase or decrease) in the thalami would be consistent with reports from histopathological examinations of brains from preterm infants with perinatal brain injury that indicate that in areas close to injured white matter regions post injury abnormal development and re-organisation of grey matter occurs, for example, neuronal changes such as atrophy, hypertrophy, changes in synaptic profiles and reactive gliosis (Marin-Padilla, 1997, 1999). In addition, these studies suggest that damage to pre-oligodendrocytes and axons may result in abnormal or disrupted thalamo-cortical connections. This might help explain the finding that in the current study thalamic abnormalities were frequently associated with widespread cortical abnormalities detected by VBM.

Both increase and decrease in grey matter density was mainly found in the datasets in which widespread VBM abnormalities were present and in those datasets with abnormalities on visual inspection of MR images, in particular in those with more severe degree of white matter reduction. Some possibilities for interpretation of these VBM results have been discussed in section 8.3.2.2.1 and 8.3.2.3.1. If one assumes that these results reflect true anatomical/biological differences between the preterms and the term controls, the finding that in the same dataset areas with increase and areas with decrease of grey matter density are present, is consistent with findings in the above mentioned studies by Allin et al (2004) and a study by Peterson et al (2003). Peterson et al (2003) found increased volume of grey matter in the frontal regions whereas in other cortical regions including the occipital grey matter and parietal grey matter, smaller volumes were found in preterms when compared to term born controls. These findings are also consistent with the results from the above mentioned neuropathology studies, i.e. that in perinatal brain injury widespread post-injury alterations with abnormal distribution of grey matter occurs.

8.4.2 VBM findings in datasets with grey matter lesions on visual inspection of MR images

In some of the datasets with widespread VBM abnormalities, grey matter lesions had already been identified by visual inspection of the MR images. In all of these datasets VBM detected additional, extra-lesional grey matter abnormalities, indicating that in those with visible focal grey matter lesions more diffuse subtle grey matter abnormalities are present that are not seem on visual inspection of the MR images. It could be speculated that in these datasets the VBM-detected subtle grey matter abnormalities are directly associated with the visible grey matter lesions, which included schizencephaly and MCA infarcts. There is evidence that, for example, malformations of cortical development such as focal cortical dysplasia of different severity, are associated with more subtle widespread grey matter abnormalities that extend beyond the visible lesion. For example, Colliot et al (2006) used single subject VBM analysis to investigate grey matter changes in patients with epilepsy and known focal cortical dysplasia. They found that VBM detected the areas of visible focal cortical dysplasia and, in addition, detected grey matter abnormalities distant from the visible lesion in 59% of the patients. Interestingly, these additional abnormalities consisted of both, increase and decrease of grey matter density. However, in the current study, it is difficult to judge whether these subtle grey matter abnormalities are secondary to a primary insult to white matter or, in fact, have to be seen in the context of primary grey matter abnormalities since in all these datasets periventricular white matter damage was also present.

8.4.3 VBM findings in the datasets with periventricular white matter lesions and no grey matter lesions on visual inspection of MR images

Of particular interest are the VBM findings in the group with visible periventricular white matter lesions but without visible grey matter lesions (i.e. without MCA infarct, malformation or cortical lesion associated with a shunt). One of the main hypotheses in this study is that in preterm children with epilepsy and/or cognitive impairment subtle (not detectable on visual inspection of MR images) grey matter abnormalities are present in addition to the white matter lesions that are typically seen in preterm children

and can be identified on visual inspection of MR images. In this study, VBM analysis was performed on 14 datasets with white matter abnormalities and no visible grey matter lesions.

Interestingly, in none of the three datasets with periventricular gliosis only (i.e. no reduction of white matter), did VBM detect any grey matter abnormalities. It is possible that injury leading to gliotic changes (i.e. the typical periventricular leukomalacia, see chapter 3, sections 3.2.1) without associated white matter reduction may occur at a later gestational age than the injury leading to white matter reduction. There is evidence that focal injury leading to gliosis occurs in more mature infants (up to term age) than haemorrhagic lesions or the diffuse form of PVL (for detailed discussion see chapter 3, section 3.2). The three children with purely gliotic changes and no VBM detected grey matter abnormalities were indeed born between 31 and 32 weeks of gestational age, i.e. at a more mature stage of brain development compared to the majority of the children in whom periventricular white matter reduction was seen. Injury at a more mature gestational age, when the neuronal organisation is more advanced, may not affect the post-injury development of grey matter in the same way as injury at a very early gestational age when neuronal organisation is less advanced. Hence the post-injury changes after insults at a more mature gestational age may not necessarily result in aberrant distribution of grey matter.

In those with periventricular white matter reduction, VBM detected grey matter abnormalities in the majority of the cases, and the number of detected grey matter abnormalities was significantly associated with the degree of white matter reduction. Possible interpretations of the findings have been discussed in section 8.3.2.3.1 and to a certain extent also in section 8.3.2.2.1. There are no other studies in preterm children that focus specifically on the investigation of subtle grey matter abnormalities in preterms with periventricular white matter reduction. The finding that widespread grey matter abnormalities consisting of both decrease and increase of grey matter density were detected in this subgroup, would be consistent with the findings from the neuropathological studies outlined in chapter 3 and above. In addition, the regions in which abnormalities were detected correspond to regions with reduced grey matter volume described in previous studies in preterm children without visible lesions or mild white matter abnormalities. However, for this subgroup too, it needs to be kept in mind

that the present lesions, some of which were large, might have led to suboptimal pre-processing of the data (e.g. misclassification of tissue during the segmentation step) so that some of the detected abnormalities are in fact artifactual findings rather than true anatomical/biological brain abnormalities.

8.4.4 Methodological considerations

There are a number of methodological issues to be considered. In this study, VBM was used with the aim of detecting subtle grey matter abnormalities in a paediatric population in which a number of subjects had visible brain lesions, mainly in the periventricular regions. The design for statistical analysis was a single subject vs group comparison and the data were smoothed with a 12 mm FWHM kernel

8.4.4.1 Normalisation and segmentation of the images

The individual MRI images were normalised to the default template (MNI template), which is an average brain created from 152 T1 weighted images obtained from adults. The choice of the template can affect the results of the VBM analysis. For example, it has been shown in recent studies in paediatric populations (e.g. Wilke, Schmithorst and Holland, 2003) that using a study specific template created from the data of the children who participated in the study, reduces the likelihood of misclassification of tissue during segmentation. In addition, the authors showed that slightly different results were obtained when comparing data normalised to an adult template with data normalised to the "paediatric" template. On the other hand, Salmond et al (2002), who investigated the effects of different templates in a study (using SPM99) that compared images of children with known hippocampal atrophy with images from healthy controls, showed that the effect of the template on the VBM results differs according to the choice of the constraints on the non-linear transformations in the normalisation process. In their study, the data were normalised once to the MNI template and then the analysis was repeated with the data normalised to a scanner and study specific template (obtained from 27 healthy children). The results were minimally dependent on the choice of the template when an optimal number of nonlinear basis functions were used in the

normalisation process. This indicates that even in paediatric populations, the MNI template is suitable when optimal normalisation parameters are used. Thus the choice of the default template in this current study is likely not to introduce a large bias into the VBM analysis.

Voxel based morphometry (using SPM software as analysis tool) has been used in previous studies in brains with gross structural abnormalities, including focal cortical dysplasia (Bonilha et al, 2006), herpes simplex encephalitis (Gitelman et al, 2001), neurodegenetative disease (Senjem et al, 2005), acquired ischaemic lesions in adults (Metha et al, 2003) and basal ganglia stroke in children (Rowan et al, 2007) and the face validity of this technique has been established. However, investigation of structural changes in grey or white matter density with VBM in brains with abnormal shape and/or gross structural abnormalities (i.e. lesions) presents special challenges, mainly due to the difficulties that arise during spatial normalisation and segmentation. For example, Shen, Szameitat and Sterr (2007) using SPM5, examined the effect of different templates using datasets with one simulated lesion. The pre-defined lesion was detected in most analyses, the detected size, however, differed for the various templates used, with the most accurate lesion delineation when a template created from healthy controls was used. They concluded that the likelihood of correctly detecting a lesion is modulated by the template but that there does not appear to be one "best" template.

Gitelman et al (2001) used VBM (in SPM99) in a group of patients who had recovered from herpes simplex encephalitis, which is known to affect specific limbic and para-limbic regions, to compare grey matter density with a healthy control group. In some of the MR images of the patients, obvious anatomical distortions were present (e.g. large necrotic changes in the temporal lobe and dilated ventricles) which, in some cases had an effect on the normalisation. The authors report VBM-detected grey matter abnormalities in the limbic and paralimbic regions in the patients, i.e. in areas that are consistent with previous histopathological studies. These abnormalities appeared to reflect the lesions seen in visual inspection of the MR images and the VBM-identified anatomical abnormalities did not overlap with visually normal cortex. Thus, these findings indicate that although obvious anatomical distortions were present in these brains, the VBM-detected areas of abnormal grey matter did not appear to be arbitrarily associated with other parts of the cortical grey matter. One additional finding in their

study was decreased grey matter density in the caudate nucleus of patients compared to the controls. However, in this study (Gitelman et al, 2001), after normalisation (which was done using a number of strategies for dealing with the presence of gross brain lesions) some residual macroscopic differences between the datasets of the patients and those of the controls were still present. In addition, the subsequent segmentation (which classifies brain tissue according to prior information about the probability of being grey or white matter based on templates that conform to normal anatomy) resulted in sub-optimal segmentation for the region of the caudate nucleus, most likely as a consequence of misclassification of tissue due to the ventricular enlargement seen in some of the patients' brains. Thus, it is not clear whether the decreased grey matter density detected in the caudate was due to true biological differences between the patients and the controls or, in fact, is attributable to a displacement of brain structures that are close to the dilated ventricles. However, this does not necessarily make inferences drawn from VBM invalid, but rather re-iterates that careful consideration of the interaction between gross brain structure and the processing and analysis methods applied in VBM is necessary and that there is no simplistic interpretation of results.

The findings of the study by Gitelman et al (2001) can be viewed as supporting the interpretation of the findings in the current study, i.e. that the VBM-detected cortical abnormalities are likely to be true anatomical/biological differences between the individual preterm brains and the control group, and that the results in the region of the basal ganglia and the thalami should be interpreted with caution, but are not necessarily invalid.

As briefly mentioned above, normalisation and segmentation of brains with lesions can be improved by a number of approaches. In the current study, the implicit brain mask was disabled for all datasets and, in datasets with large lesions, the lesions were masked. These strategies gave reasonable results in a number of the datasets in which lesions were seen. However, it has to be kept in mind that there is currently no absolute metric for the goodness of normalisation and segmentation and that judgment of successful or failed normalisation and segmentation is subjective.

8.4.4.2 Smoothing and single subject versus group analysis

Smoothing sensitises the data to a specific spatial scale that corresponds to the full width at half maximum (FWHM) of the smoothed data (see section 8.1.1.3). Thus, detection of abnormalities in small structures (e.g. the hippocampus) requires smoothing with a smaller kernel than for detection of abnormalities in larger structures (e.g. the thalamus). In this study the data were smoothed to 12 mm FWHM since in unbalanced designs such as single subject vs group comparisons smoothing to smaller kernels is likely to result in false positives (Salmond et al, 2002; for detailed discussion, see section 8.2.4). However, the trade-off when controlling for false positives in such designs, is lower sensitivity for detection of abnormalities in small structures such as the hippocampus. This is likely to explain the finding that in the majority of the datasets in which small hippocampi or signal abnormalities in the hippocampi were seen on visual inspection of images, VBM did not detect any hippocampal abnormalities.

8.5 Conclusions

In this study, VBM was used in a single subject vs group design to detect subtle grey matter abnormalities in a group of preterm children, some of which have visible brain lesions. A number of attempts have been made to adapt and optimise the standard VBM procedure to the research question and the study constraints. VBM was able to detect differences in grey matter density between individual preterm datasets and a control group, and the regions in which grey matter abnormalities were identified are consistent with findings of previous studies. Some of the findings in the datasets of brains with large lesions, however, may be caused by sub-optimal pre-processing rather than true biological differences between the preterm datasets and the controls. Thus, careful consideration of the interaction between the lesions identified on visual inspection, the pre-processing and analysis strategies applied, is necessary when interpreting the results and drawing inferences.

Part IV

Associations of neuroimaging findings with epilepsy and with cognitive function; identification of the best predictors for epilepsy and cognitive outcome

In this part of the thesis, first, associations between brain pathology as indicated by neuroimaging findings and outcome are examined. The main question to be addressed is whether in this study population there is a fundamental relationship between brain pathology as assessed by visual analysis of MR images and VBM analysis of grey matter segments, and epilepsy and overall cognitive function. Second, the imaging findings and relevant clinical variables are examined using regression analyses, with the aim of identifying the best predictors for epilepsy and cognitive function in the population of this study.

Part IV of this thesis consists of two chapters. Chapter 9 presents the results of analyses investigating associations between neuroimaging findings and epilepsy, and chapter 10 presents the results of the analyses investigating associations between neuroimaging findings and overall cognitive function. Each chapter includes a discussion of the relevant findings.

Statistics

Statistical analysis in this part of the thesis includes both univariate analyses and regression analyses. In some cases, in particular, when the numbers in subgroups were small, and for a closer inspection of associations between independent variables and the outcome variables, contingency tables were created for descriptive and detailed examination. The general approach to statistical analysis and the methods that were applied are described in chapter 4, section 4.5 and section 4.5.1.

Chapter 9: Associations between neuroimaging findings and epilepsy; examination of predictors for manifestation of epilepsy

For the analyses examining associations between findings obtained from visual inspection of MR images and outcome, datasets of 54 children (24 with epilepsy, 30 without epilepsy) were included. Data for VBM analysis were available from 45 children (17 children with epilepsy, 28 children without epilepsy; see chapter 8, section 8.2.1 and appendix 7 for details of the excluded datasets), and these 45 datasets were used to examine associations between the VBM detected grey matter abnormalities and epilepsy. Only data from children for whom both results from visual inspection of MRI and results from VBM analysis were available were included in the regression analyses.

9.1 Selection of imaging variables for examination

The main question to be answered by the analyses presented in this chapter is whether the children with a particular abnormality of white or grey matter identified by visual inspection of MR images are more likely to have epilepsy than the children without such an abnormality. Therefore, all imaging variables were retained for the analyses. With regard to the findings obtained from visual inspection of MR images (presented in chapter 7), rather than purely investigating the broad categories "presence or absence of white and/or grey matter abnormalities", for grey matter, the subcategories presence or absence of cortical, subcortical (basal ganglia, thalamus), hippocampal abnormalities, and, for white matter, the variable "white matter reduction" (with three categories: no, mild/moderate, severe) were examined. For subtle grey matter abnormalities that have been identified by VBM analysis (chapter 8), both the variable "presence or absence of VBM detected-abnormalities" and the variable "number of VBM-detected grey abnormalities" (with three categories: none=no differences in grey matter density between the dataset of a preterm child and the control datasets were seen; 1-2, "focal"=one or two peaks of differences in grey matter density between the dataset of a preterm child and the control datasets in one or different regions/structures of the brain or multiple peaks within one cluster/confined structure detected; >=3,

"widespread"=three or more peaks that were not within one cluster/confined structure detected) were chosen for examination.

9.2 Associations between brain abnormalities detected on visual inspection of MR images and epilepsy

In this section, univariate analyses investigating associations between brain abnormalities identified on visual inspection of MR images and manifestation of epilepsy and different aspects of epilepsy (such as seizure type and age at manifestation of epilepsy) are presented.

Table 9.1 shows the results of the univariate analyses for the whole study group (n=54). Nineteen (54%) of 35 children with an abnormal MRI on visual inspection had epilepsy and 16 children (46%) with abnormal MRI did not have epilepsy. On statistical testing there was only very weak evidence (Chi-square test, p=0.09) that those with an abnormality on visual assessment of MR images (without specifying the type of abnormality) were more likely to have epilepsy than those with normal MRI on visual inspection.

Table 9.1: Associations between imaging findings from visual inspection of MR images and occurrence of epilepsy in the whole study group (n=54)

MRI visual inspection (n=54 datasets)		**Epilepsy** n=24 *(% within MRI category)*	**No epilepsy** n=30 *(% within MRI category)*	**Chi-Square, Fisher's Exact, linear-by-linear association** p-value
Normal	n=19	5 *(26%)*	14 *(74%)*	
Abnormal (any abnormality)	n=35	19 *(54%)*	16 *(46%)*	0.09
White matter abnormalities (any)				
yes	n=32	18[$] *(56%)*	14 *(44%)*	
no	n=22	6 *(28%)*	16 *(72%)*	0.06**
Gliosis only				
yes	n= 5	0 -	5	n/a
no	n=49	24 *(49%)*	25 *(51%)*	
White matter reduction (+/- gliosis)				
- *no white matter reduction*	n=27	6 *(22%)*	21 *(78%)*	
- *mild/moderate*	n= 9	5 *(56%)*	4 *(44%)*	0.002 *
- *severe*	n=18	13[$] *(72%)*	5 *(28%)*	
Grey matter abnormalities (any)[$]				
yes	n=20	12[$] *(60%)*	8 *(40%)*	
no	n=34	12 *(36%)*	22 *(65%)*	0.14**
Cortical abnormalities[$]				
yes	n= 9	6[$] *(67%)*	3 *(33%)*	
no	n=35	18 *(40%)*	27 *(60%)*	0.17**
Subcortical grey matter abnormalities[$] *(basal ganglia, thalamus)*				
yes	n= 5	3 *(60%)*	2 *(40%)*	
no	n=49	21 *(43%)*	28 *(57%)*	n/a
Hippocampal abnormalities[$]				
yes	n=14	9 *(64%)*	5 *(36%)*	
no	n=40	15 *(37%)*	25 *(63%)*	0.15**
Combination of white and grey matter abnormalities[$]				0.08**
yes	n=17	11 *(65%)*	6 *(35%)*	
no	n=27	13 *(35%)*	24 *(65%)*	

MRI categories/lesions are not mutually exclusive

* linear-by-linear association used to test for trend in the frequency of the outcome across the ordered variable "degree of periventricular white matter reduction", ** Fisher's Exact test

$ includes the cases with MCA infarct (n=2), ulegyria (n=1), schizencephaly (n=1); when these cases were removed from the analysis the patterns of associations were as follows: "normal/abnormal MRI": p=0.1, "grey matter abnormalities (any)": p=0.4; "cortical abnormalities": p=0.9; "subcortical grey matter abnormalities": p=1, "hippocampal abnormalities": p=0.3, "white matter abnormalities (any): p=0.1, "degree of white matter reduction": p=0.008, "combination of grey and white matter lesions": p=0.3

9.2.1 Normal MRI on visual assessment and occurrence of epilepsy

The majority (74%) of children with normal MRI did not have epilepsy (see table 9.1 above). However, five (26%; TR, TO, TM, SSk, AP) of the children with normal MRI on visual inspection had epilepsy. In all five children age at seizure onset was after the second year of life. Judged from seizure semiology, focal onset of seizures was present in four children (with secondary generalisation in two children). One child (TR) had typical absence seizures (age of onset at eight years of age) with the corresponding typical EEG findings for absences. For four children with normal MRI and epilepsy, data from psychometric assessment were available. IQ scores, neurological status, and motor function (as indicated by the TOMI error scores) were not different from those of the 14 children without epilepsy and normal MRI on visual inspection (for IQ scores: Mann-Whitney U test, Exact; p-value for FSIQ p=0.9, for PIQ p=1, and p=0.66 for VIQ. For TOMI error scores: Mann-Whitney U test, Exact, p=0.7. For neurological status: Fisher`s Exact test, p=1).

9.2.2 Abnormal MRI on visual inspection and occurrence of epilepsy

9.2.2.1 Grey matter abnormalities

Out of the 20 children who had grey matter abnormalities, 12 (60%) had epilepsy and 8 (40%) did not have epilepsy (see table 9.1 above). Statistical testing did not indicate a significant difference (Chi-Square test, p=0.14) in the occurrence of epilepsy between those with and those without a grey matter abnormality (without specifying the type of grey matter abnormality) on visual inspection of MRI.

In both the group with and the group without epilepsy, in the majority, the presence of grey matter abnormalities was associated with periventricular white matter abnormalities. In 4 of the 12 children with grey matter abnormalities in the epilepsy group, and in 1 of the 6 children with grey matter abnormalities in the group without epilepsy, abnormalities were seen in more than one grey matter structure (i.e. cortex and hippocampi or cortex, hippocampus and basal ganglia/thalami).

Four children in the epilepsy group had grey matter lesions that are not likely to be associated with premature birth per se (MCA infarcts and malformations; for a more detailed discussion see chapter 7, section 7.7.3). Once these cases were removed from the analysis, the patterns of associations between brain abnormalities detected on visual inspection of MR images, in particular for white matter lesions, and the occurrence of epilepsy remained similar but were less strong. For the category "combination of grey and white matter lesions", for which there was weak evidence of an association with the occurrence of epilepsy before these cases were excluded, the statistical testing did not suggest any more that this particular lesion pattern was associated with epilepsy (see legend to table 9.1 above).

In the following sections, associations between abnormalities in the grey matter structures cortex, basal ganglia/thalami and hippocampi and the occurrence of epilepsy are examined in more detail. This is partly done in a descriptive manner. In addition, results from statistical testing are reported where appropriate. Regarding these statistical analyses it has to be kept in mind that the numbers in some of the grey matter categories were small. Therefore, the results obtained from the tests performed on the variables of grey matter subcategories remain tentative.

9.2.2.1.1 Cortical grey matter abnormalities

Six (JW, AM, ML, KS, SHay, HJ) of the nine children with cortical abnormalities had epilepsy (see table 9.1). Statistical testing indicated that those with cortical abnormalities were not more likely to have epilepsy than those without an abnormality in the cortical grey matter (Fisher's Exact, p=0.16). However, when examining in more detail the extent and type of the cortical lesions, in the epilepsy group, in the majority of the cases (4/6) the grey matter lesions appeared more extensive than in the group without epilepsy. Two of these four children had an MCA infarct (ML, HJ), one child (JW) had a schizencephaly and polymicrogyria, and (focal) ulegyria was seen in one child (KS). The other two children with epilepsy had small focal cortical lesions associated with a shunt (SH, AM). In contrast, in the group without epilepsy there were no children with infarcts or malformations. In this group, the cortical lesions in two of

the three children (NK, LO) consisted of small focal lesions in the context of a ventriculo-peritoneal shunt in situ, and in one child of focal cortical thinning (JC).

9.2.2.1.2 Basal ganglia and thalamus lesions

The frequency of basal ganglia and/or thalamus lesions was similar in the group with and the group without epilepsy (see table 9.1). Statistical testing was not attempted for this subcategory of grey matter lesions since the frequency in each group was very small with only three children with basal/ganglia lesions in the epilepsy group and only two children in the group without epilepsy. In the group with epilepsy, in two children (SH, KS) the abnormalities in basal ganglia and/or thalamus were associated with severe periventricular white matter reduction and abnormalities in other grey matter structures. In one child (BK) with epilepsy an isolated abnormality (high signal on T2 weighted images) in the right caudate was seen. In the two children without epilepsy (GO, RR) periventricular white matter abnormalities but no other grey matter abnormalities were seen.

9.2.2.1.3 Hippocampal abnormalities

Nine of the 14 children in whom hippocampal abnormalities (hippocampi either judged as small or signal increase on T2 weighted images) were seen on visual inspection had epilepsy (see table 9.1). Statistical testing did not indicate that hippocampal abnormalities were significantly associated with the occurrence of epilepsy (Chi Square test, p=0.15). In the group with epilepsy, in one child high signal on T2 weighted images was seen (unilaterally), in the other eight cases the hippocampi were judged as small (n=5 bilaterally, n=3 unilaterally). Similarly, in the group without epilepsy, the majority of the abnormalities were seen bilaterally (n=4; n=1 unilaterally) and all hippocampal abnormalities consisted of small hippocampi. In 4/9 (JW, ML, KS, SHay) children in the group with epilepsy and in 2/5 (NK, LO) children without epilepsy, the hippocampal abnormalities were seen in association with other grey matter abnormalities. In both groups (except in two children in the group without epilepsy)

periventricular white matter abnormalities were present on visual inspection of MR images.

9.2.2.2 White matter abnormalities

The distribution of periventricular white matter abnormalities for the group with and the group without epilepsy is shown in table 9.1 above. On statistical testing there was weak evidence that those with a white matter abnormality (either gliosis only or periventricular white matter reduction with or without gliosis) on visual inspection of MRI were more likely to have epilepsy than those without any white matter abnormality (Chi Square test, p=0.06). There was strong evidence for the occurrence of epilepsy to be more likely with more severe grades of periventricular white matter reduction (Chi square test, linear-by-linear association, p=0.002). Interestingly, no child with periventricular gliosis only had epilepsy.

Fifteen (47%) of the 32 children with periventricular white matter abnormalities (n=12 white matter reduction with or without gliosis, n=5 gliosis only) had no grey matter abnormalities on visual inspection of MRI. Seven of these children had epilepsy and eight did not have epilepsy. Figure 9.1 below shows the distribution of the white matter abnormalities in the group with and the group without epilepsy after exclusion of those with visible grey matter abnormalities. On statistical testing (after exclusion of those with grey matter abnormalities), the pattern of associations between periventricular white matter abnormalities and the occurrence of epilepsy was similar to the whole study population although less pronounced (Chi square test, linear by linear association, p=0.03).

This subgroup of children with periventricular white matter lesions but no visible grey matter lesions is of particular interest since one of the main hypotheses to be investigated in this thesis is that in those with this lesion pattern, the occurrence of epilepsy is associated with additional subtle (i.e. not identifiable on visual inspection of MRI) grey matter lesions. This is investigated in detail in section 9.3 in this chapter.

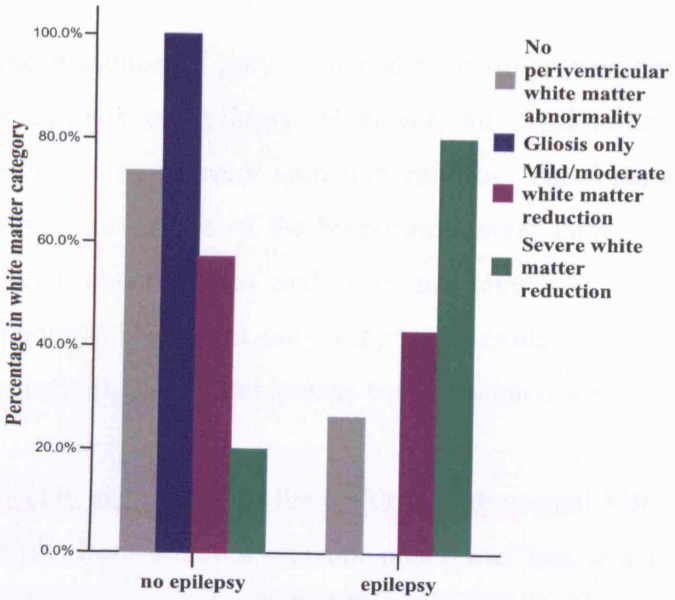


Figure 9.1: Distribution of periventricular white matter abnormalities in the group without grey matter lesions. Group without epilepsy: n=22; no white matter reduction n=17, gliosis only n=3, mild/moderate white matter reduction n=4, severe white matter reduction n=1. Group with epilepsy: n=12; no white matter reduction n=5; mild/moderate white matter reduction n=3, severe white matter reduction n=4.

9.2.2.3 Combination of white matter and grey matter lesions

In 17 (31%) of the 54 children, both white and grey matter abnormalities were seen. Eleven children (65%) of those with a combination of white and grey matter lesions had epilepsy and six (35%) did not have epilepsy. On statistical testing, there was only very weak evidence (Fisher`s Exact test, p=0.08) for the occurrence of epilepsy to be more likely in those with a combination of lesions than in those without this lesion pattern.

9.2.2.4 Summary

The results obtained from the univariate analyses showed that the degree of periventricular white matter reduction was strongly associated with the occurrence of epilepsy. No child with periventricular gliosis only (i.e. no periventricular white matter reduction) had epilepsy. The associations between degree of white matter reduction and occurrence of epilepsy remained similar once those who had associated visible grey matter abnormalities were excluded from the analysis.

The presence of grey matter abnormalities was not significantly associated with the occurrence of epilepsy. However, all children in whom cortical malformations or arterial infarcts were seen had epilepsy. Basal ganglia and/or thalamus abnormalities and abnormalities of the hippocampi were largely seen in association with other grey matter abnormalities and were not significantly associated with the occurrence of epilepsy. There was only very weak evidence that the presence of a combination of white and grey matter lesions was associated with the occurrence of epilepsy.

Twenty six percent of the children with normal MRI on visual inspection had epilepsy. In all these children, seizure onset was late and cognitive function as indicated by performance on the WISC-R and motor function was similar to those who had no epilepsy and normal MRI on visual inspection.

9.2.3 Associations between MRI findings and occurrence of neonatal seizures

As discussed in chapter 5, in the population of the current study, the occurrence of neonatal seizures has been identified as having a very strong association with the manifestation of a subsequent epileptic disorder (all children with a history of neonatal seizures developed epilepsy). This raises the question whether, with regard to the underlying brain pathology, the subgroup with neonatal seizures may represent a different group from the group of preterm children with epilepsy who did not have neonatal seizures.

In this section, first, the MRI findings in the subgroup with a history of neonatal seizures are described in more detail. Second, it is investigated whether the patterns of associations between brain abnormalities identified by visual inspection of MR images and epilepsy in the whole study group were different once the cases with neonatal seizures were excluded from analysis. To this end the univariate analyses presented above were repeated with the cases with neonatal seizures excluded.

The main findings obtained from visual assessment of MR images in the eight children with a history of neonatal seizures (WS, ML, BK, TS, HJ, JW, EG, AM) are shown in

table 9.2 below and, in more detail, and together with the neurological findings, IQ, seizure type, and EEG findings in appendix 5.

All eight children who had neonatal seizures had abnormal MRI on visual assessment (see table 9.2 below). 6/8 children in this group had more than one abnormality on visual assessment of MRI. Seven out of these eight children had white matter reduction. Six of those seven children had severe white matter reduction, and only one child had no white matter abnormality on visual assessment of MR images. In 4/8 children with neonatal seizures, large cortical abnormalities (middle cerebral infarct in two children, schizencephaly and polymicrogyria in one child) were detected on visual MRI assessment. One child had an isolated subcortical grey matter lesion (right caudate), and three children had hippocampal abnormalities (combined with cortical lesions in two cases). These findings indicate that, when compared to the children with epilepsy but no history of neonatal seizures, those with neonatal seizures have more extensive brain lesions affecting both white and grey matter. In addition, they are likely to have brain lesions that are not typically associated with preterm birth (i.e. malformations and arterial infarcts).

Table 9.2: Findings on visual analysis of MR images in the children with neonatal seizures (n=8)

ID	**MRI visual assessment: *White matter reduction***	**MRI visual assessment: *Cortical lesion***	**MRI visual assessment: *Subcortical lesion****	**MRI visual assessment: *Hippocampal lesion***
WS	mild/moderate bilateral	-	-	-
ML	severe bilateral	MCA left	-	+ small left
BK	-	-	caudate right	-
TS	severe bilateral	-	-	-
HJ	severe left	MCA left	-	-
JW	severe bilateral	schizencephaly and polymicrogyria bilateral	-	+ small bilateral
EG	severe bilateral	-	-	+ small left
AM	severe right	parietal right (shunt)	-	-

MCA= middle cerebral artery infarct; only supratentorial lesions considered
* Basal ganglia and/or thalamus lesions

After removing the eight cases with neonatal seizures from the analysis, the previously identified associations between MRI findings and the occurrence of epilepsy remained similar, although less strong. There was still a significant association between the degree of periventricular white matter reduction and the occurrence of epilepsy (Chi Square test, linear by linear association, p=0.02), whereas there was no evidence that the presence of any grey matter abnormalities (Chi Square test, p=0.7) or the combination of grey and white matter abnormalities (Fisher's Exact test, p=0.3) were significantly associated with epilepsy.

9.2.4 Associations between brain abnormalities detected on visual inspection of MR images and seizure type

The main question to be investigated in this section is whether in this study population there are any particular patterns of associations between brain abnormalities detected on visual inspection of MR images and seizure type. The analyses presented in this section are based on inspection of the frequency tables (see table 9.3 below) and are mainly of a descriptive nature. Seizures were categorised according to the categories used in chapter 5 (section 5.3.2), based on seizure semiology (i.e. seizures in which a focal onset could be identified (with or without generalisation) and seizures for which there was no suggestion for a focal origin ("only generalised seizures"). In some cases, data from interictal surface EEG were used in an attempt to improve seizure classification with regard to identifying a possible focal onset of seizure (see chapter 5). In addition, where appropriate, information on neurological status and cognitive function was included in the description of individual cases.

Table 9.3 below displays the frequencies of MR abnormalities according to seizure type. Focal seizures only were seen in 2 of the 24 children with epilepsy, focal onset seizures and generalised seizures were seen in 11/24 children (appendix 5 for details). In 4 of these 11 children, secondary generalisation after focal onset was assumed. Seizures for which, based on the available clinical data, there was no suggestion for a focal origin ("only generalised seizures") were seen in 11/24 children. Eighteen of the 24 children had more than one seizure type.

Table 9.3: Distribution of grey and white matter abnormalities identified on visual inspection of MR images in the group with focal onset seizures and the group with generalised seizures only (n=24)

MRI visual analysis **(n=24 datasets)**		**Seizures with focal onset*** **n=13**	**Only generalised seizures**** **n=11**
MRI (any abnormality)			
abnormal	n=19	9/19	10/19
normal	n= 5	4/5	1/5
White matter abnormalities (any)	n=18	9/18	9/18
White matter reduction (+/- gliosis)			
no white matter reduction	n= 6	4/6	2/6
mild/moderate	n= 5	0/5	5/5
severe	n=13	9/13	4/13
Grey matter abnormalities (any)	n=12	5/12	7/12
Cortical abnormalities	n= 6	2/6	4/6
Subcortical grey matter abnormalities (basal ganglia, thalamus)	n= 3	2/3	1/3
Hippocampal abnormalities	n= 9	5/9	4/9
Combination of grey and white matter abnormalities	n=11	5/11	6/11

Lesions are not mutually exclusive; *This group includes the subgroup with focal seizures, those with focal onset and secondary generalisation of seizures, and those with both focal and generalised seizures. ** No focal onset identified with the clinical data available.

9.2.4.1 Normal MRI on visual inspection and seizure type

Of the five children with normal MRI, four had a focal onset of seizures (TO, TM, AP, SSk). Three of the four children with focal onset seizures had complex-partial seizures, with secondary generalisation in two of these three children (TM, AP). One child (TR) with only generalised seizures (i.e. no focal onset observed) had typical childhood absence seizures with the corresponding EEG findings. None of the five children with normal MRI had clearly abnormal neurological signs (only one child had some non-specific muscular hypotonia without functional impairment). Information on cognitive

function was available on four children and FSIQ ranged from superior to low average range in this subgroup (FSIQ median 101, min 85, max 124).

9.2.4.2 Abnormal MRI on visual inspection and seizure type

Of the 19 children with abnormal findings on visual inspection of the MR images, 9 had focal onset seizures. In 8 of the 19 children both focal and generalised seizures, and in 10/19 generalised seizures only (i.e. no suggestion for a focal onset from the available clinical data) were seen.

There was no significant difference in distribution of the severity of white matter reduction between those with focal onset seizures and those in whom no focal onset could be identified (Chi Square test, linear-by-linear association, p=0.56). However, when looking in more detail at the frequency distribution of the white matter categories, it appears that those with a severe degree of white matter reduction (n=13) were more likely to have seizures with a focal onset (9/13). In contrast, in those with generalised seizures only, mild/moderate and severe periventricular white matter reduction was seen in similar frequency (n=5 mild moderate; n=4 severe). Eight of the nine children with severe white matter reduction and focal onset seizures had also generalised seizures (either secondary generalisation after focal onset or alongside focal seizures).

In those with visible grey matter abnormalities, generalised seizures only occurred at a similar frequency (n=7) to that in children with focal onset seizures (n=5). In 4/6 children with cortical lesions, based on seizure semiology no focal onset could be identified. Hippocampal abnormalities were seen in combination with other brain abnormalities and occurred at similar frequencies in both seizure type groups.

Out of the 18 children who had more than one seizure type, 14 had periventricular white matter reduction (n=11 severe, n=3 mild/moderate), 3 had normal MRI, and 1 child had an isolated lesion in the right caudate. These finding suggest that more extensive brain injury is associated with a more complex seizure disorder.

The finding that the occurrence of focal onset seizures seemed to be associated with severe white matter reduction (which indicates more widespread damage) could suggest that there might be a multifocal origin of seizures. Therefore, it was of interest to investigate the corresponding EEG data with regard to the presence of multifocal epileptic discharges in those with white matter reduction and a clinically observed focal seizure onset. There was only one child (EF) in whom multiple epileptic foci were seen on EEG. Overall, the interictal surface EEG findings were inconclusive with regard to classification of seizures and identification of a focal origin of seizures. It was also of interest to investigate whether in these children additional subtle grey matter abnormalities that might help explain the focal onset seizures could be identified with VBM. These analyses are presented and discussed in section 9.4.4 in this chapter.

9.2.4.3 Associations between brain abnormalities identified on visual inspection of MR images and the occurrence of infantile spasms

As outlined in chapter 3, several studies have reported an association between the presence of periventricular white matter lesions in preterm infants and the occurrence of infantile spasms. In the current study, four children (AM, ML, HJ, JW) had a history of infantile spasms (for clinical details see chapter 5, section 5.3.2). All four children with severe periventricular white matter reduction had cortical grey matter abnormalities on visual inspection of MRI. One child had a focal cortical abnormality associated with shunt insertion, and three children had large focal cortical abnormalities not typical of the expected pathology in preterm children (MCA infarct in two cases, schizencephaly and polymicrogyria in one case). In three children, associated hippocampal abnormalities were seen.

9.2.5 Associations between brain abnormalities detected on visual inspection of MR images and age at manifestation of epilepsy

Information on age at manifestation of epilepsy (after the neonatal period) was available for 23 of the 24 children with epilepsy (missing in one case with severe white matter reduction on MRI). Figure 9.2 shows the associations between abnormalities identified

on visual inspection of MR images and age at onset of epilepsy. There was a statistically significant difference (Mann-Whitney U test, p=0.03) in age at onset between the group with normal MRI (median 72 months, min 30, max 96) and the group with abnormalities on MRI (median 24 months, min 2, max 108). There was strong evidence for the presence of white matter abnormalities on MRI to be significantly associated with the age at epilepsy onset (Mann-Whitney U test, p=0.01). In children with no white matter reduction, median age at onset was 66 months (min 30, max 96 months).The median age at onset of epilepsy in children with white matter reduction on MRI was 24 months (min 2, max 108); (for mild/moderate white matter reduction: median age at onset of epilepsy of 18 months (min 5, max 36); for severe white matter reduction 24 months (min 2, max 108)). The presence of visible grey matter lesions was not significantly associated with age at onset of epilepsy (Mann-Whitney U test, Exact, p=0.63 for presence of any grey matter lesion). Even when the two outliers with grey matter abnormalities in the category "severe white matter reduction" and very late manifestation of epilepsy were removed from the analysis, there was still no significant association between the presence of grey matter abnormalities and age at epilepsy onset in the study population (Mann-Whitney U test, p=0.37)

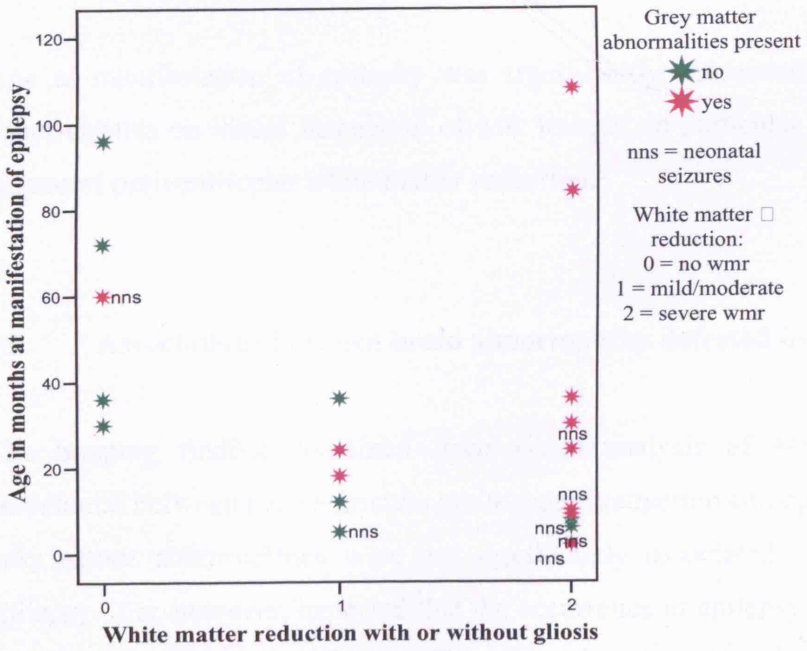


Figure 9.2: Associations between findings on visual inspection of MR images and age at manifestation of epilepsy (n=23; information on age at epilepsy onset missing in one case with severe periventricular white matter reduction). Age is corrected for gestation up to two years.

9.2.6 Summary

In sections 9.2.3, 9.2.4, and 9.2.5, the MR findings in the group with epilepsy were examined with regard to associations with the occurrence of neonatal seizures, seizure type, the occurrence of infantile spasms, and age at manifestation of epilepsy.

In the majority of the children with a history of neonatal seizures, and in all children with a history of infantile spasms, severe degrees of periventricular white matter reduction were seen. In addition, in the majority of these children, large focal grey matter abnormalities, not typical in the context of preterm brain injury, were also present.

No clear pattern of structural brain abnormalities was established for the occurrence of focal onset seizures and seizures for which no focal onset was seen. Within the subgroup of children who had periventricular white matter reduction on visual inspection of MR images, the occurrence of focal onset and presumed primary generalised seizures was similar. However, although not significant on statistical testing, it appeared that in those with focal onset seizures, the degree of white matter reduction was more severe than in those with presumed primary generalized seizures.

Age at manifestation of epilepsy was significantly associated with the presence of abnormalities on visual inspection of MR images, in particular with the presence and degree of periventricular white matter reduction.

9.3 Associations between brain abnormalities detected by VBM and epilepsy

The imaging findings obtained from visual analysis of MR images showed an association between periventricular white matter reduction and epilepsy, whereas visible grey matter abnormalities were not significantly associated with the occurrence of epilepsy. It is, however, expected that the occurrence of epilepsy is associated with grey matter abnormalities in the brain. Thus the occurrence of epilepsy is not explained by the findings from visual inspection of MR images. Since such grey matter abnormalities may be too subtle to be identified on visual inspection of MR images, a technique

(voxel-based morphometry, VBM) that has been shown to detect subtle grey matter abnormalities (see chapter 8) was employed.

The main question to be addressed in this section is whether there was an association between the VBM-detected grey matter abnormalities (see chapter 8) and the occurrence of epilepsy. Particular attention is paid to the subgroup with periventricular white matter reduction. In addition, associations between the VBM findings and aspects of epilepsy, e.g. seizure type, are discussed, mainly in a descriptive way. Where appropriate, information on neurological status and cognitive function was included in the description of subgroups or individual cases.

For the VBM analyses, 45 datasets (17 from children with epilepsy, 28 from children without epilepsy) were available and the analyses presented in this section refer to these data. For details of the datasets that were excluded from the VBM analyses, see chapter 8, section 8.2.1.

9.3.1 Subtle grey matter abnormalities detected by VBM analysis and associations with the occurrence of epilepsy in the whole group

Table 9.4 below displays the results of the univariate analyses investigating findings from VBM analysis of grey matter and the occurrence of epilepsy for the whole group (n=45).

On statistical analysis there was only weak evidence that the presence of VBM-detected grey matter abnormalities was associated with the occurrence of epilepsy (Fisher's Exact, p=0.06). There was, however, a strong association between the number of detected grey matter abnormalities and the occurrence of epilepsy (Chi Square test, linear by linear association, p=0.01).

Table 9.4: Associations between grey matter abnormalities detected by VBM analysis and occurrence of epilepsy in the whole group (n=45)

VBM abnormalities **(n=45 datasets)**	**Epilepsy** **n=17** ***(%within VBM category)***	**No epilepsy** **n=28** ***(%within VBM category)***	**Chi-Square, Fisher's Exact, linear-by-linear association** **p -value**
VBM grey matter abnormalities detected n=28	14 *(50%)*	14 *(50%)*	0.06*
No VBM grey matter abnormalities detected n=17	3 *(18%)*	14 *(82%)*	
Number of VBM-detected grey matter abnormalities			
0 n=17	3/17 *(18%)*	14/17 *(82%)*	
1-2 ("focal") n=16	6/16 *(38%)*	10/16 *(63%)*	0.01**
>=3 ("widespread") n=12	8/12 *(67%)*	4/12 *(33%)*	

VBM= voxel-based morphometry; classification of VBM-detected grey matter abnormalities (for details see chapter 8, section 8.2.6.): 0: no difference in grey matter density between the dataset of a preterm child and the datasets of the control group detected; 1-2 ("focal"): one or two peaks of differences in grey matter density between the dataset of a preterm child and the control datasets or multiple peaks within one cluster/confined structure detected; >=3 ("widespread"): three or more peaks that were not within one cluster/confined structure.

* Linear-by-linear association used to test for trend in the frequency of the outcome across the ordered variable "number of VBM abnormalities"

** Fisher's Exact test

9.3.2 Subtle grey matter abnormalities detected by VBM analysis and associations with epilepsy – group with normal MRI on visual inspection

Table 9.5 below shows the results from VBM analysis of grey matter segments for the subgroup with normal MRI on visual inspection (n=18; n=4 children with epilepsy, n=14 children without epilepsy) and associations with the occurrence of epilepsy. VBM analysis detected abnormalities in nine children (n=2 children (TM, SSk) with epilepsy, n=7 (CS, MD, ASa, SDaW, BA, SF, JRu) children without epilepsy). In all cases, except one (JRu), the VBM-detected grey matter abnormalities were focal. There was no suggestion from inspection of the frequency tables and from statistical analysis that in those with normal MRI the presence of VBM-detected grey matter abnormalities was associated with the occurrence of epilepsy (Fisher's Exact, p=1). This result from

statistical analysis, however, has to be regarded with caution since the groups were very unbalanced and the numbers small.

Table 9.5: Associations between grey matter abnormalities detected by VBM analysis and occurrence of epilepsy in the subgroup with normal MRI on visual inspection (n=18)

VBM abnormalities (n=18 datasets)		**Epilepsy** n=4	**No epilepsy** n=14
VBM grey matter abnormalities detected	n=9	2/9	7/9
No VBM grey matter abnormalities detected	n=9	2/9	7/9
Number of VBM-detected grey matter abnormalities			
0	n=9	2/9	7/9
1-2 ("focal")	n=8	2/8	6/8
>= 3 ("widespread")	n=1	-	1/1

VBM= voxel-based morphometry; classification of VBM-detected grey matter abnormalities (for details see chapter 8, section 8.2.6.): 0: no difference in grey matter density between the dataset of a preterm child and the datasets of the control group detected abnormalities; 1-2 ("focal"): one or two peaks of differences in grey matter density between the dataset of a preterm child and the control datasets in one or different regions/structures of the brain or multiple peaks within one cluster/confined structure detected; >=3 ("widespread"): three or more peaks that were not within one cluster/confined structure.

In the two children with epilepsy and focal VBM abnormalities (TM, SSk), the locations of the detected grey matter abnormalities were in the insular cortex (TM) and the cingulate gyrus (SSk). In both children, focal and generalised seizures were seen. In the child with abnormalities in the cingulate gyrus, some of the observed clinical symptoms (fear, vocalisation and slurred speech) were compatible with frontal lobe seizures. In the child with abnormalities in the insular cortex, a detailed description of the focal seizures was not available. Both children had normal interictal surface EEGs. In both children, no abnormal neurological findings (CP) were seen. For one (SSk) of the two children, results from psychometric assessments were available and these were in the average to high average range.

In two children (TR, TO) with normal MRI on visual inspection and epilepsy, no grey matter abnormalities were detected by VBM. One child (TR) had typical simple absence seizures with the typical EEG abnormalities. The other child (TO) had complex-partial seizures and generalised epileptic discharges on EEG. Both children had late epilepsy onset (TO age 6 years, TR age 8 years), no abnormal neurological findings (CP), and performance on the WISC-R was in the low average to average range.

9.3.3 Subtle grey matter abnormalities detected by VBM analysis and associations with epilepsy – group with abnormal MRI on visual inspection

Table 9.6 below shows the results from VBM analysis of grey matter segments and associations with the occurrence of epilepsy for the subgroup with abnormal MRI on visual inspection. VBM analysis detected grey matter abnormalities in 19 (12/13 children with epilepsy, 7/14 children without epilepsy) of the 27 children with abnormal MRI on visual inspection. Focal grey matter abnormalities (i.e. 1-2 peaks) were detected in four children (LB, BK, TC, SH) in the group with epilepsy and in four children (GO, BBe, DSk, NK) in the group without epilepsy. More widespread abnormalities (i.e. >=3 peaks) were detected in eight children in the epilepsy group and in three children (CO, LO, DHay) in the group without epilepsy.

The results obtained from statistical testing indicated that those with abnormal MRI and grey matter abnormalities detected by VBM were more likely to have epilepsy (Fisher's Exact, p=0.03). In addition, the results suggested that there was a significant association between the number of VBM-detected grey matter abnormalities and the occurrence of epilepsy (Chi square test, linear by linear association, p=0.01).

Table 9.6: Associations between grey matter abnormalities detected by VBM analysis and occurrence of epilepsy in the subgroup with abnormal MRI on visual inspection (n=27)

VBM abnormalities **(n=27 datasets)**	**Epilepsy** **n=13**	**No epilepsy** **n=14**	**Chi-Square, Fisher's Exact, linear-by-linear association** **p-value**
VBM grey matter abnormalities detected n=19	12/19	7/19	0.03**
No VBM grey matter abnormalities detected n=8	1/8	7/8	
Number of VBM-detected grey matter abnormalities			
0 n= 8	1/8	7/8	
1-2 ("focal") n= 8	4/8	4/8	0.01*
>= 3 ("widespread") n=11	8/11	3/11	

VBM= voxel-based morphometry; classification of VBM-detected grey matter abnormalities (for details see chapter 8, section 8.2.6) 0: no difference in grey matter density between the dataset of a preterm child and the datasets of the control group detected abnormalities; 1-2 ("focal"): one or two peaks of differences in grey matter density between the dataset of a preterm child and the control datasets in one or different regions/structures of the brain or multiple peaks within one cluster/confined structure detected; >=3 ("widespread"): three or more peaks that were not within one cluster/confined structure.

* linear-by-linear association used to test for trend in the frequency of the outcome across the ordered variable "number of VBM abnormalities"

** Fisher's Exact test

In seven children with epilepsy and in six children without epilepsy, grey matter lesions were seen on visual inspection of MRI. VBM detected grey matter abnormalities in excess of the visible grey matter lesion in 5/7 children (ML, AU, BK, JW, AM) in the group with epilepsy. In the group without epilepsy, VBM detected grey matter abnormalities in addition to the visible grey matter abnormalities in 3/6 (BBe, GO, LO) children. In the group with epilepsy, the additional grey matter abnormalities detected by VBM were widespread in all except one child, whereas in the group without epilepsy they were focal in all except in one child. These findings, albeit a small number of children, further support the suggestion that in the epilepsy group grey matter damage is more extensive and the distribution more widespread, possibly predisposing to being at greater risk for epilepsy.

9.3.3.1 Subtle grey matter abnormalities detected by VBM analysis and associations with epilepsy – group with periventricular white matter lesions and no grey matter abnormalities on visual inspection of MRI

This section focuses on the subgroup of children with "pure" periventricular white matter lesions (i.e. the cases in which associated grey matter abnormalities were detected on visual inspection of MR images were excluded). In section 9.2.2.2 it was shown that after exclusion of those with visible grey matter abnormalities, periventricular white matter reduction was still significantly associated with the occurrence of epilepsy. Thus this subgroup with "pure" periventricular white matter lesions is of particular interest with regard to more detailed investigation of the contribution of VBM for detection of subtle grey matter abnormalities that may be associated with the occurrence of epilepsy in preterm children with white matter injury.

Twenty four of the 32 datasets in which periventricular white matter lesions (either gliosis only or white matter reduction with or without gliosis) were identified by visual inspection of images were available for the VBM analysis. In 14 of these 24 datasets (n=11 white matter reduction, n=3 gliosis only), no grey matter abnormalities were seen on visual inspection. Six of these children had epilepsy (TC, SH, PD, WS, LR, EF) and eight (MC, LiWi, LWa, NSi, LH, DSk, DHay, CO) did not have epilepsy.

Figure 9.3 below displays the distribution of the VBM-detected grey matter abnormalities in the subgroup with periventricular white matter lesions and no associated visible grey matter lesions. Similarly to the whole group (n=45), statistical testing indicated that the occurrence of epilepsy was associated with the presence of VBM-detected grey matter abnormalities (Fisher's Exact p=0.03). In addition, there was an indication that those with a greater number of VBM-detected grey matter abnormalities were more likely to have epilepsy (Chi Square test, linear by linear association p=0.04). Although the numbers in the individual categories were small and the results from statistical testing have to be treated with caution, the distribution suggests a pattern similar to the pattern that was seen for the association between the occurrence of epilepsy and the degree of periventricular white matter reduction, in that the occurrence of epilepsy was associated with more severe lesions, here indicated by the number (categorised as either 1-2 or >3 as defined above) of VBM-detected subtle

grey matter abnormalities. Indeed, there was a positive correlation between the degree of white matter reduction and the number of VBM-detected grey matter abnormalities (see chapter 8 for a detailed discussion).

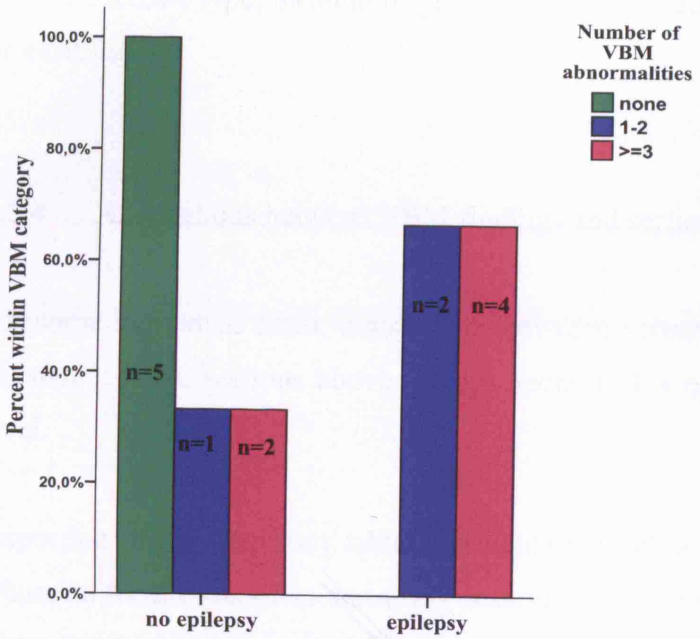


Figure 9.3: VBM-detected grey matter abnormalities in the group with periventricular white matter abnormalities and no visible grey matter lesions (n=14). Group without epilepsy: n=8 (gliosis only, no white matter reduction n=3, mild/moderate white matter reduction n=4, severe white matter reduction n=1). Group with epilepsy: n=6 (mild/moderate white matter reduction n=3, severe white matter reduction n=3).

In the two children with epilepsy, periventricular white matter reduction and focal VBM abnormalities, the locations of the VBM-detected grey matter abnormalities were in the right parietal lobe (TC) and the cingulate gyri bilaterally (SH). In the child with abnormalities in the cingulate gyri, the clinical symptoms (shaking of left arm, eye deviation, non-responsive) and EEG findings (epileptic discharges right parietal) were not typical for frontal lobe seizures. The other child with a grey matter abnormality in the right parietal lobe (TC) had, based on clinical signs, no focal onset of seizures and no focal epileptic discharges on interictal surface EEG. Both children performed in the average range on the WISC-R.

In two (EF, LR) of the four children (PD, WS, EF, LR) with epilepsy, periventricular white matter reduction and widespread VBM abnormalities, focal and generalised seizures were seen; the other two children (WS, PD) had generalised seizures. Overall, for this subgroup of children, for whom the VBM findings suggested more widespread subtle grey matter lesions in addition to the white matter injury, no clear relationship between seizure type, location of grey matter abnormalities, and/or EEG findings could be established.

9.3.4 Associations between VBM findings and seizure type

For some individual cases, associations between seizure type and VBM findings were discussed in the sections above. In this section, this question is explored on a group level.

Inspection of the frequency tables (see table 9.7 below) might suggest that in those in whom no focal onset of seizures was observed (i.e. in those who had presumed primary generalised seizures), more widespread VBM-detected grey matter abnormalities were seen when compared to those with focal onset seizures. However, the numbers in the cells were very small and thus these results have to be regarded as exploratory.

A systematic investigation of the contribution of clinical signs, interictal surface EEG findings and location of VBM abnormalities to determining the location of a seizure focus was not attempted, since it was felt that the available data were not sufficient for such an analysis.

Table 9.7: Distribution of VBM-detected grey matter abnormalities in the group with focal onset seizures and the group in which no focal onset was identified (generalised seizures only) n=17

VBM abnormalities **(n=17 datasets)**	**Focal seizures only** **n=1**	**Focal and generalised seizures** **n=6**	**Generalised seizures only** **n=10**
Number of VBM-detected grey matter abnormalities*			
0	1	-	2
1-2 ("focal")	-	3	3
>= 3 ("widespread")	-	3	5

VBM= voxel-based morphometry; classification of VBM-detected grey matter abnormalities (for details see chapter 8, section 8.2.6.): 0: no difference in grey matter density between the dataset of a preterm child and the datasets of the control group detected abnormalities; 1-2 ("focal"): one or two peaks of differences in grey matter density between the dataset of a preterm child and the control datasets in one or different regions/structures of the brain or multiple peaks within one cluster/confined structure detected; >=3 ("widespread"): three or more peaks that were not within one cluster/confined structure.

9.3.4.1 Subtle grey matter abnormalities detected by VBM analysis and associations with the occurrence of infantile spasms

MR datasets from three (AM, ML, JW) of the four children who had a history of infantile spasms were included in the VBM analysis. In all three cases VBM analysis detected widespread grey matter abnormalities in addition to the white and grey matter abnormalities seen on visual inspection of MR images There was, however, no consistent pattern recognisable in the location of the VBM-detected grey matter abnormalities in this subgroup with infantile spasms.

9.3.5 Subtle grey matter abnormalities detected by VBM analysis and associations with the occurrence of neonatal seizures

In five (WS, ML, BK, JW, AM) of the eight datasets from children who had a history of neonatal seizures, VBM analysis was performed. Three (AM, ML, JW) of these

children also had a history of infantile spasms. In four of the five cases, widespread grey matter abnormalities were detected by VBM and in only one child (BK), the VBM-detected grey matter abnormalities were focal, i.e. confined to one structure. In all children, VBM detected additional grey matter abnormalities in areas other than those identified on visual inspection. Only two children (BK, AM) had VBM detected grey matter abnormalities confined to the cortical grey matter whereas in all other cases a combination of cortical, cerebellar and thalamic abnormalities was detected. It is noteworthy that all children with neonatal seizures (except one, BK) and all children with infantile spasms had periventricular white matter reduction on visual inspection of MRI.

9.3.6 Summary

The results obtained from univariate analyses showed that in the whole group (i.e. all for whom VBM analysis was performed, n=45) the presence of VBM-detected grey matter abnormalities was only weakly associated with the occurrence of epilepsy. However, there was a strong association between the number of detected VBM grey matter abnormalities and the occurrence of epilepsy, i.e. the presence of more widespread VBM-detected grey matter abnormalities was strongly associated with epilepsy. This suggests that in the preterm children with epilepsy, when compared to those without epilepsy, more severe and widespread brain injury, that includes grey matter in addition to periventricular white matter injury that is typically seen in preterm children, is present.

In the group of children who had normal MRI on visual inspection, the presence of VBM-detected grey matter abnormalities was not significantly associated with the occurrence of epilepsy. The analysis in this subgroup was, however, limited by small numbers and unbalanced groups; thus this result remains tentative. In one of the two children with normal MRI, epilepsy, and focal VBM-detected grey matter abnormalities, the location of the grey matter abnormality was compatible with the seizure semiology.

In the group of children with abnormal MRI on visual inspection, the presence of VBM-detected grey matter abnormalities was significantly associated with the occurrence of seizures. Furthermore, the number of the detected VBM grey matter abnormalities showed a strong association with epilepsy. The associations between VBM-detected subtle grey matter abnormalities and the occurrence of epilepsy remained similar once only those with periventricular white matter lesions and no grey matter lesions on visual inspection of MR images were included in the analysis.

In the subgroup with epilepsy and grey matter lesions detected on visual inspection of MRI, VBM analysis detected additional subtle grey matter abnormalities in areas other than those in which grey matter lesions were seen on purely visual inspection of MRI more frequently than in those without epilepsy. This suggests that in the children with visible grey matter lesions and epilepsy, grey matter damage is present that is more widespread than in those without epilepsy, and only in parts identifiable on visual inspection of MR images.

There was a suggestion from inspection of frequency tables that in those children in whom no focal onset of seizures was observed (i.e. those with generalised seizures only), more widespread VBM-detected grey matter abnormalities were seen. However, examination of the data with regard to associations between seizure type and VBM findings on a group level was limited and remains inconclusive.

In the children with a history of infantile spasms and in children with a history of neonatal seizures, the findings obtained from the VBM analysis further support the findings obtained from analysis performed on the data from the visual inspection of MR images, i.e. that in these preterm children extensive brain damage was present, involving both white and grey matter.

9.4 Regression analyses

In this section, the results of the logistic regression analyses are presented. The main questions to be answered by these analyses were first, which of the available imaging variables best predicted epilepsy, and second, whether VBM grey matter analysis added clinically useful predictive information to findings obtained from visual inspection of MR imaged in the study population. Finally, an analysis was performed in which some relevant clinical variables (see chapter 5, section 5.5.1 for selection of variables of interest) were entered in the regression model together with the imaging variables.

Given the relatively small study sample, the main aim of the logistic regression analyses was to determine which of the available variables provide clinically useful prognostic information without attempting to quantify risk or infer causation. Therefore the values of significance levels, adjusted odds ratio, and 95% confidence intervals are used mainly as guides to point to possible predictors of epilepsy. The overall approach regarding the statistical analyses employed in this study is discussed in chapter 4, section 4.5.

To avoid overlooking important associations, the approach was liberal with regard to inclusion of variables in the regression analyses since, due to the likely complex inter-relationships among variables, they might contribute to a regression in unexpected ways. Variables were entered one at a time and the contribution of each variable to the explanation of the outcome variable was assessed at each step.

The following imaging variables were examined: degree of white matter reduction on visual inspection of MRI, grey matter abnormalities identified on visual inspection of MRI, presence of VBM-detected grey matter abnormalities, number of detected VBM grey matter abnormalities (i.e. 0, 1-2=focal, >=3=widespread). Perinatal and neonatal variables of interest were then entered into a second set of regression analyses. Similarly, to the analyses performed in chapter 6, correlation matrices were created to identify correlations between independent perinatal and neonatal variables. Based on these correlation matrices, principal component scores (PCAs) were calculated for the correlated independent peri- and neonatal variables gestational age, birth weight, ultrasound category, APGAR at 5 minutes, duration of oxygen supplementation and

persistent ductus arteriosus (PDA).The first two sets of derived variables, namely "PCA_1" (mainly dominated by birth weight, gestational age and by duration of oxygen supplementation) and "PCA_2" (dominated by ultrasound diagnosis, secondly by PDA and APGAR at 5 minutes) from this reduced dataset were entered in the regression model. Gender was also included in the analyses.

The univariate analyses identified the occurrence of neonatal seizures as a very strong risk factor for epilepsy (see chapter 5). All children with a history of neonatal seizures developed epilepsy and no child in the group without epilepsy had a history of neonatal seizures. Thus the variable "neonatal seizures" in this sample is very deterministic and this subgroup with neonatal seizures does not add new information when investigating the predictive value of imaging findings for epilepsy, which was the main question to be addressed with the regression analyses. In addition, the distribution of the variable "neonatal seizures" in the study sample results in an empty cell in the 2*2 contingency tables and consequently this variable can not be entered in a regression model (no maximum likelihood exists for this variable). Therefore it was decided to examine the predictive value of the imaging and clinical variables with regression analyses on a reduced subset that does not include the cases with a history of neonatal seizures, and to investigate the group with neonatal seizures in detail with univariate and descriptive analyses with regard to associations with clinical variables and the underlying brain pathology (these results are presented in chapter 5, section 5.6. and in this chapter in section 9.3.5 above).

Data from children of whom a complete set of data (i.e. results from visual inspection of MR images and results from VBM analyses) was available and who did not have a history of neonatal seizures were included in the analyses. Of the 45 children (n=17 with epilepsy, n=28 without epilepsy) with a complete dataset, 5 had neonatal seizures (ML, WS, BK, JW, AMB). Thus, data from 40 children (28 children without epilepsy, 12 children with epilepsy) were included.

9.4.1 Results from the regression analyses

Table 9.8 below shows regression coefficients, standard errors, estimated odds ratios, 95 % confidence intervals and p-values.

The regression model was built up manually with the variable "degree of white matter reduction" entered first, which explained 21% of the variation in diagnosed epilepsy. When "grey matter abnormalities identified on visual inspection" was entered as the second variable, only minimal improvement in prediction of outcome was achieved. Therefore this variable was not retained in the model. The variable "presence of VBM-detected grey matter abnormalities" was added next (coefficients, standard error, estimated odds ratio, 95% confidence intervals and p-values are shown in table 5.7 below), which led to an improvement in the rate of prediction (from 73% when only "degree of white matter reduction" was in the model, to 78%), and this model with the two variables "degree of white matter reduction" and "presence of VBM-detected grey matter abnormalities" explained 24% of the variance in the outcome. The estimated odds ratio for those with VBM detected grey matter abnormalities was 2.2, indicating an increased risk for epilepsy when compared to those with no VBM-detected grey matter abnormalities. However, this was not significant (p=0.3, see table 9.9) and with this variable in the model the effect of the variable "degree of white matter reduction" was weaker. It was decided to explore whether entering the variable "number of VBM-detected grey matter abnormalities" instead of "presence of VBM-detected grey matter abnormalities" might improve the model. However, entering this variable resulted in a non-ignorable increase in the standard errors, i.e. the model became less precise and less stable. This was most probably caused by the variables "degree of white matter reduction" and the "number of VBM-detected grey matter abnormalities" having some degree of collinearity (the degree of white matter reduction and the number of detected VBM peaks was correlated, see chapter 8) and thus sharing variation. Therefore this variable was removed from the model. Table 9.8 below shows the coefficients, standard error, estimated odds ratio, 95% confidence intervals and p-values for the final model. In this model only the variable "degree of white matter reduction" (with the categories none, mild/moderate, severe) was retained since this provided the most stable model with the best goodness of fit. Based on this model the adjusted odds ratio for epilepsy for those with a mild/moderate degree of white matter reduction was 5.2 (p=0.06). The

odds ratio for epilepsy for those with a severe degree of white matter reduction was 7.0 (p=0.04). Adding the clinical variables (PCA_1, PCA_2, gender) to the model with white matter reduction as the only retained imaging variable did not improve the prediction of epilepsy.

In summary, the regression analyses exploring which of the available variables provided the best predictor for epilepsy in this study population, indicated that periventricular white matter reduction identified on visual inspection was the best single predictor. The inclusion of findings from VBM grey matter analyses did not substantially improve the prediction of epilepsy over visual inspection of MR images and using the degree of periventricular white matter reduction as single predictor. The clinical data that were explored in these regression analyses did not contribute to improvement of prediction of epilepsy in the examined population over and above the examined imaging variables.

Table 9.8: Final model that only retained the variable "degree of periventricular white matter reduction"

	B	**SE**	**Adjusted OR**	**95% CI**	**p-value**
Periventricular white matter reduction					
Mild/moderate white matter reduction	1.7	0.89	5.2	0.9-30.2	0.06
Severe white matter reduction	1.9	0.93	7.0	1.1-44.1	0.04

B= coefficient; SE=standard error; OR=odds ratio; CI=confidence interval

Table 9.9: Model that retained the variables "degree of periventricular white matter reduction" and "presence of VBM-detected grey matter abnormalities"

	B	SE	Adjusted OR	95% CI	p-value
Periventricular white matter reduction					
Mild/moderate white matter reduction	1.56	0.91	4.9	0.83-29.1	0.07
Severe white matter reduction	1.71	0.97	5.5	0.82-36.9	0.07
Presence of VBM-detected grey matter abnormalities	0.77	0.83	2.2	0.42-11.1	0.3

B= coefficient; SE=standard error; OR=odds ratio; CI=confidence interval

9.5 Discussion

The main objective of the analyses presented in this chapter was to investigate associations between brain pathology as indicated by qualitative (visual inspection of MR images) and quantitative (VBM analysis of grey matter segments) neuroimaging findings and epilepsy. One of the main questions to be addressed was whether VBM-detected grey matter abnormalities are associated with epilepsy in this cohort of preterm children in whom the expected brain pathology typically consists of injury to the white matter. Second, regression analyses including neuroimaging variables and clinical variables were performed with the aim of identifying the best predictors of epilepsy in the study population.

All of the 54 MR datasets were used for the analyses examining associations between findings from visual inspection of MR images and epilepsy. For the analyses investigating associations between VBM-detected grey matter abnormalities and epilepsy, 45 datasets were used. As described in detail in chapter 8, section 8.2.1, six datasets had to be excluded from VBM analysis since one or more steps in the pre-processing gave poor results. In all these excluded datasets, large periventricular white matter lesions were seen, which were combined with visible grey matter abnormalities in five cases. Five of the six children had epilepsy. It has to be kept in mind when

interpreting the results of some of the analyses investigating the relationships between VBM-detected grey matter abnormalities and epilepsy that the exclusion of these datasets might have introduced a bias.

For both investigation of univariate associations between findings from visual inspection of MR images with epilepsy and investigation of univariate associations between findings from VBM and epilepsy, in some analyses, the numbers were small and thus the results remain tentative. It also has to be kept in mind that the regression analyses presented in this chapter have not been performed with the aim of identifying causative factors but rather to determine which of the available variables provided clinically useful information for prediction of epilepsy in the study population under investigation.

9.5.1 Neuroimaging findings and associations with the occurrence of epilepsy

The analyses presented in this chapter showed that the presence of periventricular white matter reduction was significantly associated with the occurrence of epilepsy, whereas the presence of visible grey matter abnormalities was not significantly associated with epilepsy. However, VBM analysis of grey matter segments identified subtle grey matter abnormalities in a number of the datasets and these abnormalities were significantly associated with the occurrence of epilepsy. These results suggest that in those preterm children who have epilepsy in addition to the typical injury to the periventricular white matter, additional subtle grey matter lesions are present and that these present a neuroanatomical correlate of epilepsy.

The degree of periventricular white matter reduction was significantly associated with epilepsy, i.e. those with a severe degree of white matter reduction were more likely to have epilepsy than those with a mild/moderate degree of white matter reduction.

There are no studies available that use qualitative and quantitative MRI to investigate neuropathological correlates of epilepsy in preterm children without selecting the study participants for either the presence of periventricular white matter injury and/or presence of cerebral palsy. Thus comparison of the findings from the current study with

the existing literature is limited. However, keeping this in mind, some of the main findings from existing studies that investigate the associations between the severity of periventricular white matter lesions and epilepsy in preterm children pre-selected for the presence of periventricular lesions and/or cerebral palsy are comparable and consistent with the main findings obtained from visual inspection of MR images in the current study. For example, Humphreys et al (2007), investigated a population of children (n=154; 40 preterm children, 29 of whom had epilepsy) with cerebral palsy subtypes that are expected to be associated with bilateral periventricular leukomalacia (i.e. leg-dominated bilateral spastic cerebral palsy). Imaging consisted of MRI, CT, or, in some cases, of neonatal cranial ultrasound, and periventricular lesions were categorised into four grades, with grade four including focal grey matter abnormalities in addition to periventricular white matter lesions. In their study, the occurrence of epilepsy was significantly associated with the extent of the periventricular white matter lesion. The authors acknowledge that in those with epilepsy, small focal grey matter lesions may be present in addition to the white matter damage, but that due to the retrospective nature of the study, the limited sensitivity of CT and ultrasound for detection of subtle lesions, and the limitation of imaging analysis to visual inspection of images, a detailed investigation of this issue was not possible. Only a minority (n=9) of the children with epilepsy had visible focal cortical lesions detected on MRI and no information on gestational age at delivery was given on these children. Although the main findings of their study are consistent with the results of the current study, it has to be noted that, in the study by Humphreys et al (2007), the exclusion of children with other types of cerebral palsy than bilateral forms and the different imaging modalities used for investigation of brain abnormalities might have introduced a bias into the analyses. In a morphometric study that primarily aimed at investigating associations between lateral ventricular volume on MRI (as an indirect sign of periventricular white matter loss) with cognitive and motor impairment in a group of children with bilateral cerebral palsy and periventricular leukomalacia (cases with signs of intraventricular haemorrhage and/or visible grey matter lesions on MRI were excluded), Melhem et al (2000), found that there was no significant difference in mean lateral volume between those with and those without epilepsy. The authors interpreted their findings such that, in those with epilepsy, concomitant subtle cortical malformations that have no influence on the measured ventricular volumes and are undetectable on MR images are present. For better comparison of the current study with the Melhem et al study, an additional

analysis was done within the subgroup who had cerebral palsy, in which the (subjectively judged) degree of white matter reduction was compared between those with and those without epilepsy. Similarly to the study by Melhem et al (2000), there was no significant difference in the severity of reduction of white matter between the two groups (Chi Square test, linear by linear association, p=0.16); there was, however, a significant difference between those with and those without epilepsy with regard to the presence of VBM-detected grey matter abnormalities (Fisher's Exact test, p=0.02). Although there are differences in the methods of assessment for white matter injury between the two studies, and in the current study children with other types than only bilateral CP were included and the numbers in these additional analysis were very small, these findings nevertheless can be regarded as further support for the hypothesis that within the group with periventricular white matter abnormalities, additional subtle grey matter injury is present that may explain the occurrence of epilepsy.

In the whole group, VBM analysis detected additional subtle brain abnormalities in the grey matter more frequently in those with epilepsy and white matter reduction than in those without epilepsy and white matter reduction. In addition, the VBM-detected grey matter abnormalities in those with epilepsy were more widespread than the VBM-detected grey matter abnormalities in those without epilepsy, indicating that the injury to grey matter is more severe in those preterm children who have epilepsy. It could be speculated that in those with epilepsy, a "threshold" regarding the severity of grey matter injury may have been crossed. The occurrence of epilepsy might be considered as a reflection of the severity of brain damage in this group of children. The finding that there was a tendency for those with periventricular white matter reduction and epilepsy, in particular, in the subgroup with a severe degree of white matter reduction, to perform worse on the assessments examining overall cognitive function (Full Scale IQ; Mann-Whitney U test, p=0.06) and neuromotor function (TOMI error scores; Mann-Whitney U test, p=0.004), and that the proportion of children with the diagnosis of CP was higher (no epilepsy 4/9, epilepsy 14/19) in this group when compared to those with white matter reduction and no epilepsy, might provide further support for the interpretation of more extensive brain damage being present in those with epilepsy. In addition, the findings in the subgroup of children with periventricular gliosis only and no white matter reduction on visual inspection of the MR images support this notion.

None of these children with gliosis only had epilepsy and only two of these children had VBM-detected grey matter abnormalities (which were focal).

Interestingly, when comparison was made between those with and those without epilepsy within the group with severe white matter reduction, different patterns with regard to the grey matter abnormalities identified on visual inspection of MR images and VBM-detected grey matter abnormalities seemed to emerge. Although the proportions of those with visible grey matter abnormalities in both groups were comparable (4/5 of those without epilepsy and 9/13 of those with epilepsy had visible grey matter abnormalities), in the group with epilepsy in contrast to those without epilepsy, in the majority of the cases the VBM-detected subtle grey matter abnormalities were widespread. However, this finding has to be interpreted with caution. The numbers in these subgroups were very small and unbalanced between subgroups, and thus the results remain tentative and require further investigation in groups with larger sample sizes. In addition, in the group with epilepsy and severe white matter reduction, the visible grey matter lesions included large focal lesions such as malformations and MCA infarcts, i.e. lesions that affect large grey matter areas and are know to carry a high risk for epilepsy (see e.g. Kuzniecky, 2006, for brain malformations; Golomb et al, 2007, for MCA infarcts). The VBM-detected grey matter lesions and epilepsy in this subgroup might not necessarily be attributed to injury sustained in the context of preterm birth and primary damage to white matter with secondary subtle grey matter abnormalities (see also chapter 8, section 8.4.2). However, since in all these preterm children with MCA infarcts or malformation severe periventricular white matter reduction in the typical distribution seen in preterm brain injury was present as well, it is difficult to assess whether the VBM-detected subtle grey matter abnormalities are to be seen primarily in the context of the visible large focal grey matter lesions or in the context of preterm brain injury. The interpretation that the widespread VBM-detected grey matter abnormalities in those with epilepsy are a consequence of preterm brain injury might be supported by the finding that the patterns of associations between epilepsy and VBM findings in the children with white matter reduction remained similar once the cases with visible grey matter lesions had been excluded from the analysis.

Neither for abnormalities in the basal ganglia/thalami nor abnormalities in the hippocampi was there a suggestion for an association with the occurrence of epilepsy. It would have been of interest to explore in more detail whether in the group with epilepsy hippocampal sclerosis was more frequent than in the group without epilepsy. In order to make a diagnosis of hippocampal sclerosis from neuroimaging data, it has been suggested that two of the following criteria should be met: abnormal high signal on T2 weighted images, decreased signal on Inversion Recovery T1weighted images, atrophy of the hippocampus, and loss of internal structure of the hippocampus (Jackson et al, 1993). These criteria have become widely accepted across centres with epilepsy surgery programs. However, the imaging protocol of the current study did not allow such detailed assessment of the hippocampus. In both the group with and the group without epilepsy, hippocampal abnormalities were judged mainly as being small; abnormal high signal on T2 weighted images was observed only in one case in the epilepsy group. The lack of association between imaging abnormalities and epilepsy in the present study may be due to the limited range of imaging findings in this preterm group. However, hippocampal abnormalities are frequently seen in preterm children, and it would be of great interest to investigate in greater detail whether in preterm children with epilepsy, the range and type of abnormalities in the hippocampus are different from preterm infants without epilepsy.

Overall, the findings from the analyses presented in this chapter, in addition to the data presented in chapter 7 and 8, are consistent with the findings from the neuropathological studies described in chapter 3, sections 3.2.3.2 and 3.2.3.3, and further discussed in chapter 8, section 8.4. These studies, mainly the work by Marin-Padilla (1996, 1997 and 2000), suggest that in the brains of infants with large haemorrhagic white matter lesions, alterations of the microstructure of the primarily undamaged grey matter overlying the areas of damaged white matter can be present. These changes in grey matter have been termed by Marin-Padilla as "acquired cortical dysplasia" and it has been suggested that these histopathological findings are likely to explain some of the neurological impairments and/or epilepsy that were detected in the brains of the infants who had undergone these post-mortem histopathological examinations. Furthermore, it seems plausible that brain injury in the context of preterm birth can lead to destruction of subplate neurons and subsequent altered cortical development (see e.g. McQuillen et al, 2003; Volpe, 1996). It seems plausible that the VBM-detected grey matter abnormalities

are a reflection of these abnormalities in cortical grey matter that are likely to predispose to epilepsy.

There are no other studies available that specifically investigate the associations between VBM-detected subtle grey matter lesions and epilepsy in preterm children. In general, studies in children using VBM for investigation of structural correlates of epilepsy are rare. However, in a recent study, Cormack et al (2005), using VBM for investigation of grey matter in term-born children with temporal lobe epilepsy and hippocampal sclerosis (and no other visible lesions on MR images), found reduced grey matter ipsilateral to the seizure focus not only in the hippocampus, but also widespread bilaterally in the temporal and extra-temporal regions and frontal and parietal cortices, i.e. in regions compatible with anatomical distribution of hippocampal connections. The authors speculate that in addition to brain damage caused by early onset seizures, grey matter loss in early onset temporal lobe epilepsy may be caused by abnormal cortical development following loss of functional input from the sclerotic hippocampus. One might speculate and regard this secondary abnormal cortical development following primary injury to the hippocampus as a similar biological phenomenon to the presumed secondary developmental grey matter abnormalities after primary white matter injury in preterm infants.

In adults, several studies using VBM for investigation of grey matter abnormalities in different types of epilepsy, and for both normal MR images on visual inspection and MR images in which areas of focal cortical dysplasia were seen, have shown that additional, often widespread, subtle grey matter abnormalities were present. For example, Bernasconi et al (2004), using VBM in a large sample of adults with temporal lobe epilepsy, found grey matter abnormalities beyond the hippocampus, involving other limbic areas such as the cingulum, thalamus and extra-limbic areas. Similarly, Keller et al (2002a), found widespread VBM-detected grey matter abnormalities in patients with temporal lobe epilepsy, involving prefrontal, thalamic and cerebellar regions. Interestingly, in their study, increase as well as decrease of grey matter density in different brain regions was found. In the current study, too, in particular in those with widespread abnormalities, both decrease and increase of grey matter density was found (see also chapter 8, section 8.4.1).

In adults with idiopathic generalised epilepsy, who, by definition, have normal MRI on visual inspection, specifically in patients with absence epilepsy (Betting et al, 2006) and with juvenile myoclinic epilepsy (Woermann et al, 1999), VBM studies have shown widespread regional cortical grey matter abnormalities, mainly with increase in mesio-frontal regions, the thalamus (absence epilepsy; Betting et al, 2006) and in frontal basal regions (juvenile myoclonic epilepsy; Woermann et al, 1998 and 1999; Betting, et al, 2006). In the current study, a number of children with epilepsy had normal MRI on visual inspection. Only two of these five children had seizures that could be interpreted as being within the spectrum of the idiopathic generalised epilepsies. In only one of these two children VBM detected subtle grey matter abnormalities, which were focal and not widespread. These findings seem inconsistent with the results from the above mentioned studies. There are several possible explanations for this inconsistency: in the current study, there was only a small number of children with epilepsy and no obvious lesions on MR images, and within this small group several different types of seizures were seen with only a minority suggesting that they may be within the spectrum of idiopathic generalised epilepsies. The classification of seizures was mainly based on seizure semiology with information obtained from the parents. Thus in some cases the seizure types and classification of epilepsy syndrome was not straightforward and may be not correct. Second, in contrast to the above-mentioned studies, in the current study, the design of the VBM analysis was a single subject – group comparison. In addition, different amounts of smoothing were used in the above studies (ranging from 5mm to 12 mm) compared to the current study (12 mm). These technical details might partly explain the difference in VBM findings. Finally, in the current study, which focuses on a selected population in which a specific type of brain injury is expected (with the injury primarily affecting the white matter), the underlying brain pathology might be different from that in the above-mentioned studies in which most likely not many patients who have a history of preterm birth were included.

There are several studies (e.g. Woermann et al, 1999; Bonilha et al, 2006) that investigate grey matter in detail using VBM in adults with partial seizures and focal cortical dysplasia (FCD), i.e. patients who have visible grey matter abnormalities on MR images. Keeping in mind that, in contrast to the current study, these studies investigated data from adults, and did not have the technical difficulties caused by the presence of abnormally shaped brains and e.g. large ventricles, overall the findings from

the current study are consistent with findings from these studies. For example, Bonilha et al (2006) examined a small sample of patients with drug-refractory partial epilepsy caused by FCD with an adapted version of VBM ("optimised VBM") in a single subject-group comparison design. They found in the majority of the patients, in addition to the regions that had been identified as abnormal by visual inspection, regions of abnormal grey matter density in other brain areas different from visually detected FCD areas. Interestingly, in their study, in some patients, the VBM-detected grey matter abnormalities were scattered over more than one cerebral lobe and all these patients had multifocal ictal or interictal EEG abnormalities. Two patients in this study by Bonilha et al (2006) had neurological findings other than seizures (hemiparesis in one case and learning difficulties in the other case) and both patients had extensive VBM-detected grey matter abnormalities.

9.5.2 Neuroimaging findings and seizure type

In the current study, it was not possible to define a pattern of associations between the imaging findings, seizure type (categorised as focal onset seizures and seizures in which no focal onset could be determined), and interictal EEG findings on a group level. When exploring possible associations between findings from visual inspection of MR images and seizure type, it appeared that focal onset seizures were more frequent in those with a severe degree of white matter reduction whereas in those in whom no focal onset could be identified, both mild/moderate and severe reduction of white matter occurred in similar frequency (see section 9.2.4.3). However, on statistical testing there was no significant difference with regard to the severity of white matter reduction between the two seizure type groups. With regard to the VBM-detected subtle grey matter abnormalities, there was an indication that widespread abnormalities were more frequent in those with a focal seizure onset. EEG findings however, did not indicate a possible multifocal origin of seizures in these cases.

Overall, these findings are inconclusive and there are a number of reasons that are likely to have limited this analysis. The numbers in the groups were very small, seizure classification was based on the clinical description, which might have resulted in missing focal onset in seizures with rapid generalisation or in missing subtle focal signs,

and interictal surface EEG was used, which has limited value in localisation of a seizure focus.

9.5.3 Age at onset of epilepsy

Age at onset of epilepsy was significantly associated with the severity of white matter reduction but not with the presence of grey matter abnormalities on visual inspection of MR images. There have been previous reports that suggest that the extent of periventricular lesions and age at onset of epilepsy show a negative relation (for review, see e.g. Aicardi, 1994). When examining VBM-detected subtle grey matter abnormalities for more detailed assessment of the presence and extent of subtle grey matter damage and associations with age at onset of epilepsy, interestingly, the results indicated that there was a weak suggestion for an association for both the presence and numbers of VBM-detected grey matter abnormalities with age at onset of epilepsy (presence of VBM abnormalities: Mann-Whitney U test, $p=0.09$. Number of VBM abnormalities: Spearman's $\rho=0.3$, $p=0.05$). Other studies have found strong associations between age at onset of epilepsy and VBM-detected grey matter abnormalities in specific brain regions. For example, Keller et al (2002 b), in a study in adults with temporal lobe epilepsy using regression analyses, found associations between the age at onset of epilepsy and VBM-detected grey matter abnormalities in the thalamus, cerebellum, and prefrontal areas. In the current study, no attempt was made to investigate associations between age at onset of epilepsy and regional distribution of VBM-detected subtle grey matter abnormalities; nevertheless, the findings in both studies indicate that age at onset of epilepsy is negatively related to the extent of subtle grey matter damage.

9.5.4 Neuroimaging findings and infantile spasms

Infantile spasms in the population of the current study were seen in the context of extensive brain lesions, involving both periventricular white matter and cortical grey matter, detected on visual inspection of MR images and VBM analysis. All children, except one, with a history of infantile spasms had large focal grey matter lesions

including infarcts and, in one case, a brain malformation. Thus, in addition to the severe white matter abnormalities seen in all these children, different types of brain lesions were present in this small subgroup with a history of infantile spasms. It is well described that in those with infantile spasms of symptomatic etiology, a range of different brain abnormalities are seen, which are often of prenatal origin and extensive, including brain malformations (Riikonen, 1984) and focal cortical dysplasia (Guerrini et al, 1996). There are several studies that have found an association between periventricular white matter lesions and infantile spasms. For example, Okumura et al (1996), investigated a group of infants with periventricular leukomalacia (defined as either abnormally high signal on T2 weighted images in the periventricular white matter and/or large ventricles with irregular margins) and found that, in all the infants who developed infantile spasms, severe periventricular lesions (defined as marked ventriculomegaly extending to the frontal horns of the lateral ventricles) were present, whereas none of those with mild periventricular lesions had infantile spasms. Ozawa et al (1998), in a morphometric study in preterm infants with periventricular leukomalacia (defined in a similar way as in the study by Okumura et al, 1996), found that in those with infantile spasms, in addition to the periventricular lesions, atrophy in the pons and midbrain was present when compared to those without infantile spasms and a control group without periventricular leukomalacia. In the current study, pons and midbrain were not specifically assessed. The finding in the above-mentioned studies, i.e. that there is an association between the severity of the periventricular white matter reduction and infantile spasms, is compatible with the results from the current study. However, in none of the above-mentioned studies did the authors comment on grey matter, and thus it is not possible to judge whether some of the infants had, in fact, also grey matter lesions that might be associated with infantile spasms, similarly to those found in the current study.

9.5.5 Neuroimaging findings and neonatal seizures

A history of neonatal seizures was strongly associated with later epilepsy in this study population (see also chapter 5, section 5.5.). Three of the eight children in this subgroup also had infantile spasms. All children with a history of neonatal seizures, except one, had extensive periventricular white matter reduction on visual inspection of MRI, and

half of the children in this group had visible large focal cortical grey matter lesions. VBM analysis detected widespread grey matter abnormalities in six of the eight children, involving cortical, thalamic and cerebellar grey matter. These findings suggest that in this subgroup severe brain damage was present and suggest that the occurrence of neonatal seizures can be regarded as a marker for the severity of the brain injury. It is well described (see e.g. Volpe 2001; Tekgul et al, 2006) that global hypoxic-ischaemic injury, focal ischaemic injury (e.g. arterial infarcts such as MCA infarcts), intracranial haemorrhage, brain malformations, central nervous system infections, and transient metabolic disturbances, or inborn errors of metabolism are associated with neonatal seizures. In preterm children, intracranial haemorrhage has been identified as a common cause of neonatal seizures (see e.g. Volpe, 2001). The MRI findings from visual assessment of white matter in the current study are compatible with a history of haemorrhage early in life in the majority of the children neonatal seizures in this study population. However, for a preterm population, there was a surprisingly high proportion of children who also had lesions of different origin such as MCA infarcts and, in one case, brain malformation.

9.5.6 Regression analyses

On of the main questions that were investigated in this chapter, was which of the available data would provide the best predictor for epilepsy in this study population. For the imaging variables, a particular question was whether the VBM grey matter findings would add additional prognostic information over purely visual inspection of MR images. It must be emphasised that the regression analyses were not performed with the aim of determining causation or quantifying risk. Rather these analyses were performed to identify variables or a set of variables that provide clinically useful prognostic information.

Although the confidence intervals were large and thus the results can not be considered very precise, the results from the regression analyses nevertheless suggest that the best predictor in this dataset was the presence of periventricular white matter reduction, in particular, when the degree of white matter reduction was severe (adjusted OR 7.0). It is noteworthy that, even those in which the degree of white matter reduction was judged as

mild/moderate, the risk was considerable with an adjusted OR of 5.5. Interestingly, the presence of visible grey matter abnormalities on visual inspection of MRI did not improve the prediction of epilepsy when entered together with white matter reduction into the model. VBM-detected grey matter abnormalities appeared to present an increased risk for epilepsy (adjusted OR 2.2); however, adding this information to the regression model did not significantly improve the prediction of epilepsy. When some clinical variables that were regarded as relevant (see chapter 5, section 5.5.1) were added to the model, no improvement of prediction was achieved. This suggests that superimposed on clinical variables, there is an independent effect of brain injury as indicated by neuroimaging findings, and that an absolute relationship exists between the detected brain lesions and epilepsy.

Similar limitations to those mentioned in the context of the univariate analyses apply when comparing the findings from the regression analysis with the existing literature. Most studies that used regression analyses investigated risk factors for childhood epilepsy in general. There is no study that uses regression analysis and focuses on preterm children except the study by Humphreys et al (2007). However, in their study, children were enrolled on the basis of a diagnosis of periventricular leukomalacia and there was a substantial number of term born children in the sample. In contrast to the current hospital-based study in which it was not attempted to quantify risk or infer causation, most studies that have used regression analysis focused on identifying and quantifying risk factors for childhood epilepsy and are population-based.

In most of the existing population-based studies on risk factors for childhood epilepsy, brain malformations, intracranial haemorrhages, a history of neonatal seizures, and a positive family history of epilepsy have been identified as strong risk factors for epilepsy (see e.g. Nelson and Ellenberg, 1986; Whitehead et al, 2006; Humphreys et al, 2007). The majority of the existing studies did not have the tools and sufficiently detailed data to allow investigation of the brain in such detail as in the current study, and none of the existing studies used quantitative MRI analysis methods for detection of subtle grey matter abnormalities. Thus the results from the regression analysis in the current study examining the neuroimaging variables potentially add important information.

In the current study, in the univariate analyses, neonatal seizures had been identified as having a very strong association with later epilepsy. However, in the regression analyses, the independent contribution of a positive history of neonatal seizures to prediction of later epilepsy could not be examined, since all children with neonatal seizures developed epilepsy, resulting in this variable being deterministic, and thus no maximum likelihood could be estimated for this particular variable (see section 9.4 in this chapter).

9.5.7 Methodological considerations and limitations of the analyses performed

There are a number of methodological issues and some limitations regarding the statistical analyses that have to be considered in the interpretation of the data presented in this chapter.

First, in some of the univariate analyses, in particular when specific questions were investigated requiring splitting the whole study group into subgroups, the numbers were small and thus the results from these analyses need to be interpreted with this in mind. Second, MR datasets from six children (5/6 with epilepsy) with large periventricular white matter lesions and abnormally shaped brains had to be excluded from the VBM analyses (see chapter 8, section 8.2.1 and section 8.4). The exclusion of these datasets, resulting in missing information on potentially present subtle grey matter lesions in these datasets, might introduce a bias into both the univariate and regression analysis that examine the associations between VBM-detected brain abnormalities and epilepsy. Third, regression analyses were performed on a subset of data with the datasets from children who had a history of neonatal seizures excluded.

Visual analyses of MR images is subjective and this needs to be kept in mind when interpreting the results of the univariate and regression analyses. However, there was good agreement between the two raters who scored the MR findings (though formal inter-rater reliability testing was not performed). In chapter 8 (section 8.4.4), some methodological and technical issues regarding the VBM analyses have been discussed in detail. One important technical issue that needs to be considered when interpreting the findings is, that there is a likelihood that some of the VBM findings, in particular, in

those datasets in which large visible lesions and abnormally shaped brains were present, may not reflect true biological grey matter abnormalities but rather be artefacts of image processing. Thus VBM findings from such datasets might have to be regarded as less reliable. However, in the majority of the datasets in which visible grey matter abnormalities were present, VBM analysis confirmed these visible lesions. This indicates that the VBM-detected abnormalities that were seen in excess of visible lesions in those datasets with large visible lesions and abnormally shaped brains are, in fact, likely to represent true biological differences in grey matter between preterm children and the control group. In addition, the main findings from the VBM analysis of grey matter segments in this study are compatible with existing studies in other preterm populations (for detailed discussion see chapter 8, section 8.4), which provides further support for the assumption that the VBM-detected grey matter abnormalities reflect true anatomical abnormalities and are not entirely caused by technical artefacts. The segmented data were smoothed with an 12 mm Gaussian kernel, which results in low sensitivity for detection of abnormalities in small structures such as the hippocampus (see chapter 8, section 8.4.4.2), a structure that is of interest when investigating neuroanatomical correlates of epilepsy. Thus in this study, the performed VBM analysis most likely missed some hippocampal abnormalities that might be present in this study population.

Overall the examination of the data with regard to associations between seizure types and neuroimaging findings was limited and the exploratory analyses on a group level remained inconclusive. A detailed examination of relationships between seizure types, EEG findings and neuroimaging findings was limited for several reasons. First, seizure types were classified based mainly on seizure semiology and information on clinical signs at onset and during seizures was obtained by parental interview. In addition, only interictal surface EEG data were collected. Clinical observation and retrospectively collected data on clinical signs of seizures might result in missing important details that are necessary for an accurate seizure classification. For example, a focal onset of seizures with fast secondary generalisation might be missed. Interictal surface EEG data are not very precise at localisation of a seizure focus. Thus the current study can not be regarded as a definite study as far as investigation of associations between seizure types and qualitative and quantitative neuroimaging findings is concerned.

9.6 Conclusions

The analyses performed for the investigation of associations between qualitative and quantitative neuroimaging findings and the occurrence of epilepsy identified strong associations of epilepsy with reduction of periventricular white matter and with VBM-detected subtle grey matter abnormalities, in particular widespread VBM abnormalities. These findings suggest that in the children with epilepsy, when compared to those without epilepsy, subtle, often widespread, grey matter abnormalities are present that are likely to predispose to epilepsy.

The analyses presented in this chapter suggest that in this sample the best predictor for epilepsy was the degree of periventricular white matter reduction identified on visual inspection of MR images. The presence of VBM-detected subtle grey matter abnormalities presented an increased risk for epilepsy. However, using information on the presence of subtle grey matter abnormalities identified by VBM analysis did not significantly improve the prediction of epilepsy over visually assessing the degree of periventricular white matter reduction on MR images.

A number of methodological issues that may influence the data and analyses need to be considered and some findings of this study should be regarded as tentative, in particular those that investigate specific aspects of epilepsy such as seizure type. Further work regarding these issues and larger scale studies are required.

Chapter 10: Associations between neuroimaging findings and cognitive function; examination of predictors for overall cognitive outcome

In this chapter, first, results from univariate analyses that examine associations between neuroimaging findings and overall cognitive function as indicated by performance on the Wechsler Intelligence Scale for Children-Revised (WISC-R) are described. In addition, exploratory analyses are performed to examine whether in this group of preterm children, epilepsy has an independent effect on cognitive function once brain lesions are accounted for. Second, results from regression analyses are presented that examine whether information obtained from neuroimaging further improves prediction of overall cognitive outcome in addition to those clinical variables that have been identified in the analyses described in chapter 6 as providing clinically useful prognostic information.

It has to be kept in mind that the investigation of cognitive function in this study is limited to the two subscales of the WISC-R, PIQ and VIQ, and that detailed investigation of associations between specific cognitive deficits and structural brain abnormalities is not possible with the data available.

All imaging variables were retained for these analyses. With regard to the findings obtained from visual inspection of MR images (presented in chapter 7), rather than purely investigating the broad categories "presence or absence of white and/or grey matter abnormalities", for grey matter, the subcategories presence or absence of cortical, subcortical (basal ganglia, thalamus), hippocampal abnormalities, and, for white matter, the variable "white matter reduction" (with three categories: no, mild/moderate, severe) were examined, too. For subtle grey matter abnormalities that have been identified by VBM analysis (chapter 8), both the variable" presence or absence of VBM detected-abnormalities" and the variable "number of VBM-detected grey abnormalities" (with three categories: none = no differences in grey matter density between the dataset of a preterm child and the control datasets were seen; 1-2, "focal" = one or two peaks of differences in grey matter density between the dataset of a preterm child and the control datasets in one or different regions/structures of the brain or multiple peaks within one

cluster/confined structure detected; >=3, "widespread" = three or more peaks that were not within one cluster/confined structure detected) were chosen for examination.

Two children (TM, epilepsy and JC, no epilepsy) of the original group of 54 children did not have a psychometric assessment and thus were not included in the analyses. Eight further children (ML, AU, SHay, TS, HJ, JW, EG, EF, all had epilepsy) were excluded from the analyses because the severity of their motor impairments did not allow them to complete the required number of tests on the verbal and/or performance subscales needed for computation of an IQ score. Thus, for the analyses examining associations between findings from visual analysis of MR images and performance on the WISC-R, datasets from 44 children (15 with epilepsy and 29 without epilepsy) were included. A detailed description of those children's clinical data and comparison of relevant clinical data between those for whom psychometric data were available and those for whom no psychometric data were available is given in chapter 6, section 6.1. Details on findings obtained from visual inspection of MR images are given in table 7.2 chapter 7.

Data for VBM analysis were available for 45 children (17 with epilepsy and 28 without epilepsy); from the original group of 54 children, for three children (TS, AP, JC) no 3D MPRAGE datasets were available, and pre-processing of the 3D MPRAGE datasets from six children (CT, KS, SHay, HJ, EG, PP) was unsuccessful, i.e. these datasets had to be excluded from further analysis (see chapter 8, section 8.2.1). For 5 (TM, ML, AU, JW, EF, all had epilepsy) of the 45 children from whom VBM data were available, no data from psychometric testing were available. Thus, in the analyses investigating associations between findings from VBM grey matter analysis and cognitive function, 40 datasets were included.

Only data from children for whom data from visual inspection of MR images, results from VBM analysis and psychometric data were available, were included in the final regression analyses (n=40).

10.1 Associations between findings on visual inspection of MR images and cognitive outcome as indicated by performance on the WISC-R

Table 10.1 below shows, for the whole group of 44 children and for each MRI category, the median PIQ and median VIQ with minimum and maximum, and, where appropriate, results from statistical testing investigating associations between imaging findings and IQ scores. The lesion categories are not mutually exclusive. In some of the categories the numbers were small (cortical abnormalities n=4, subcortical abnormalities n=4, gliosis only n=5); therefore examination of these variables is limited to descriptive analyses.

Table 10.1: Median PIQ and VIQ scores in each MRI category and associations between MRI findings and IQ scores (n=44)

MRI visual inspection (n=44 datasets)		**Performance IQ** *Median (min-max)*	*p-value**,**	**Verbal IQ** *Median (min-max)*	*p-value**,**
Normal	n=18	96.5 *(74-119)*		104.5 *(77-131)*	
Abnormal (any abnormality)	n=26	69 *(45-109)*	**0.001***	89 *(57-119)*	**0.008***
White matter abnormalities (any)	n=23	74 *(45-109)*	**0.02***	96 *(57-119)*	**0.12***
Gliosis only	n= 5	96 *(57-100)*	**n/a**	102 *(87-115)*	**n/a**
Periventricular white matter reduction (+/- gliosis)			**0.004****		**0.03****
- mild/moderate	n= 9	95 *(45-109)*		90 *(67-119)*	
- severe	n= 9	56 *(46-101)*		86 *(57-110)*	
Grey matter abnormalities (any)	n=13	68 *(46-101)*	**0.01***	80 *(57-115)*	**0.007***
Cortical abnormalities	n= 4	60 *(46-75)*	**n/a**	82 *(57-103)*	**n/a**
Subcortical abn. (basal ganglia, thalamus)	n= 4	84.5 *(46-101)*	**n/a**	78 *(57-115)*	**n/a**
Hippocampal abn.	n= 9	61 *(46-95)*	**0.007***	80 *(57-110)*	**0.02***
Combination of white and grey matter abnormalities	n=10	71 *(46-101)*	**0.1***	88 *(57-115)*	**0.09***

MRI categories are not mutually exclusive
* Mann-Whitney U test, Exact
** Spearman Rank test

There was a significant difference between those without and those with abnormal findings on visual inspection of MR images in both median PIQ (Mann-Whitney U test, Exact, p=0.001) and VIQ (Mann-Whitney U test, Exact, p=0.008).

In the group of children with normal MRI on visual inspection (n=18), the majority had scores in or above the average range for both PIQ (12/18) and VIQ (16/18). Six children had PIQ scores in the low average or borderline range. VIQ scores in the low average or borderline range were seen in two children. Four (22%) out of the 18 children had epilepsy. The majority of the children with normal MRI had normal (11/18; 61%) or suspicious (7/18, 39%) neurological findings and no child had cerebral palsy.

In the group with abnormal findings on MR images there was a larger variation than in the group with normal MRI for both IQ scales (see table 10.1). Although the IQ profile was similar in both groups, with the VIQ being higher than the PIQ, the differences between PIQ and VIQ were more pronounced in the group with abnormal MRI. In contrast to the group with normal MRI, the majority of children in the group with abnormal MRI findings had PIQ scores below average. Sixteen of the 26 children in the group with abnormal findings on MRI had PIQ scores in the borderline or below borderline range and only 9 had scores in the average range. One child had a PIQ in the low average range. In contrast, VIQ seemed better preserved, with half of the 26 children having VIQ scores in the average or high average range, 4 in the low average range, and 9 in the borderline/below borderline range. In contrast to the group with normal MRI, only 4/26 (15%) had normal neurological findings; 12/26 (46%) had suspicious findings, and 10/26 (38%) had cerebral palsy. Ten out of the 26 children (38%) had epilepsy.

In the majority of the cases, abnormalities were seen bilaterally. In only five datasets, clearly unilateral lesions were seen on visual inspection of MR images (n=3 left, n=2 right). Therefore it was felt that it was not meaningful to formally examine the effect of side of lesion on IQ scores in the context of this study.

10.1.1 Periventricular white matter abnormalities and associations with Performance IQ and Verbal IQ

The presence of periventricular white matter abnormalities (i.e. periventricular gliosis only or periventricular white matter reduction with or without gliosis) showed a significant association with PIQ only (Mann-Whitney U test, Exact, p=0.02; see table 10.1).

In the subcategories of white matter abnormalities, for those with periventricular gliosis only, both median PIQ and VIQ were in the average range. Only two children (NS, NK) had PIQs in the below borderline range (NS: PIQ 57) and low average range (NK: PIQ 75) respectively. One of these two children (NK) had, in addition to the periventricular gliosis, a small cortical lesion in the left temporal lobe and small hippocampi bilaterally. Verbal IQ in this child was in the average range (VIQ 100), while in the other child (NS), who had abnormal neurological findings (cerebral palsy), both PIQ and VIQ were reduced (PIQ 57, VIQ 87).The other three children had IQ scores in the average to high average range. However, in these children, too, there was a discrepancy between PIQ and VIQ of greater than five IQ points, with the PIQ being lower than the VIQ.

In the subgroup with periventricular white matter reduction, the degree of white matter reduction showed a significant negative correlation with both PIQ (Spearman's ρ= - 0.43, p=0.004) and VIQ (Spearman's ρ= - 0.33, p=0.03).

Of the nine children with a mild/moderate degree of white matter reduction, only two (SD, LB) had additional grey matter abnormalities, which were small and confined to one structure. In both children, these consisted of small hippocampi. In contrast, in the group with severe white matter reduction, six out of the nine children had additional grey matter abnormalities. These consisted of cortical lesions in three cases (LO, KS, AM), basal ganglia/thalamus lesions in two cases (RR, KS), and abnormalities of the hippocampi in four cases (CT, LO, KS, PP). Two of the six children had abnormalities in more than one grey matter structure (KS, LO).

In order to explore whether in the group with periventricular white matter abnormalities, the additional grey matter abnormalities might affect the associations with IQ scores,

the analysis was repeated excluding the datasets in which additional (to the periventricular white matter lesions) grey matter lesions were seen excluded. This reduced dataset consisted of 18 MRI data sets without and 13 MRI data sets with periventricular white matter abnormalities. The associations between the presence of periventricular white matter abnormalities remained similar with evidence for an association with PIQ only, albeit weak (Mann-Whitney U test, Exact, p=0.05 for PIQ; p=0.15 for VIQ). However, after exclusion of those with additional grey matter abnormalities, only for PIQ did a significant correlation with the severity of white matter reduction remain (Spearman's ρ= -0.4, p=0.03). For VIQ no significant correlation with the degree of white matter reduction and VIQ was seen (Spearman's ρ= -0.2, p=0.18).

In 18 (41%) of the 44 children, the corpus callosum was judged as thin (generally or part of the corpus callosum), and in all but one child (BK) this was combined with periventricular white matter abnormalities (n=3 gliosis only, n=6 mild/moderate white matter reduction, n=8 severe white matter reduction). Within the group of those with periventricular white matter lesions (without taking the degree of white matter reduction into account), when IQ scores were compared between those with (n=17) and those without (n=7) thinning of the corpus callosum, the analysis suggested a significant difference for both PIQ (Mann-Whitney U test, p=0.002) and VIQ (Mann-Whitney U test, p=0.01).

10.1.2 Grey matter abnormalities and associations with Performance and Verbal IQ

For both PIQ and VIQ, statistical testing showed evidence for an association with the presence of grey matter abnormalities (not specified which structures affected) on visual inspection of MR images (Mann-Whitney U test, Exact, p=0.01 for PIQ, and p=0.007 for VIQ; see table 10.1 above).

In the subgroup with grey matter lesions, when datasets with white matter lesions were excluded, three datasets, in which only grey matter lesions were seen on visual inspection of images, remained (BBe, AT, BK). Two of these three children had small hippocampi bilaterally and one child had a lesion in the right caudate (BK).

Performance IQ scores in these three children were in the borderline or below borderline range, VIQ scores ranged from low average to below average range. One of the three children (BK) had epilepsy.

Abnormalities in the hippocampi were significantly associated with both PIQ (Mann-Whitney-U test, Exact, p=0.007) and VIQ (Mann-Whitney U test, Exact, p=0.02, see table 10.1 above). However, hippocampal abnormalities were mainly seen in combination with white matter lesions (see chapter 7 for detailed discussion). In only two cases (BBe, AT) isolated hippocampal abnormalities were seen. Therefore, similarly to the other grey matter abnormalities such as cortical or subcortical (i.e. basal ganglia and/or thalamus) abnormalities, it was not possible to examine the effect of isolated lesions in these structures in more detail. However, the overall frequency of hippocampal abnormalities was high enough to allow for testing whether the presence of such abnormalities in the group with periventricular white matter abnormalities might affect IQ scores. When comparison was made between those who had white matter lesions with hippocampal abnormalities (n=7) and those who had white matter lesions without hippocampal abnormalities (n=16), there was no evidence for a difference in IQ scores between these two groups (Mann-Whitney U test, Exact, p=0.4 for PIQ, and p=0.2 for VIQ), which may indicate that in those with periventricular white matter reduction, additional hippocampal abnormalities have no significant effect on PIQ and VIQ.

The numbers in the grey matter subgroups basal ganglia/thalamus abnormalities and cortical abnormalities were too small for formal statistical testing (see table 10.1); therefore, the analyses concerning these structures are dealt with in a purely descriptive way. All cortical abnormalities were seen in combination with periventricular white matter abnormalities (severe white matter reduction in three cases, LO, KS, AM; and in one case gliosis only, NK). In three cases (KS, LO, NK) abnormalities in other grey matter structures were present as well. Performance IQ ranged from below borderline (KS, AM) to borderline (NK, LO). Verbal IQ ranged from below borderline (KS, AM) to average (NK, LO). When comparison was made for median IQ scores between those with cortical lesions (n=4) and those without cortical lesions (n=6) taking account of the periventricular white matter reduction, there was a difference in median PIQ of 12 points (for those with cortical lesions: median 50, min 46, max 75, for those without

cortical lesions: median 62, min 52, max 101), and for VIQ, there was a 9 point difference (for those with cortical lesions: median 82, min 57, max 103; for those without cortical lesions: median 91, min 60, max 110). However, it has to be kept in mind that the numbers in the two groups were very small and that, as mentioned above, in both groups abnormalities in other grey matter structures were seen in a proportion of children. Thus, based on the available data, it remains difficult to draw conclusions about the direct effect of cortical lesions on cognitive outcome as indicated by performance on the WISC-R.

In four children abnormalities in the basal ganglia and/or thalami were seen on visual inspection of MR images (see table 10.1 above). Only one child had isolated basal ganglia lesions (BK). Three of the four children had periventricular white matter lesions (GO, RR, KS). IQ scores in the group with basal ganglia/thalamus abnormalities ranged from below borderline to high average and, based on the four cases in this subgroup, no specific pattern between IQ scores and the presence of basal ganglia/thalamus lesions emerged.

In the original group of 54 children, there were four children who had brain abnormalities that are not typical of preterm brain injury, e.g. middle cerebral artery infarcts or malformations. Except one child with ulegyria (KS), none of these children were able to complete the required number of subtests necessary for calculation of IQ scores and thus are in the group of excluded children. This might introduce a bias into the investigation of associations between grey matter abnormalities and IQ scores, since in these children grey matter abnormalities consisted of large cortical lesions combined with abnormalities in other grey matter structures and in periventricular white matter.

10.1.3 Combination of grey and white matter abnormalities and associations with IQ scores

In 10 children visual inspection of MR images identified both periventricular white matter lesions and grey matter lesions. In addition to the analyses described above, which partly already address the question of the effect of a combination of grey and white matter lesions on cognitive function in this study group on a descriptive level, it

was investigated more formally and on a group level, whether the presence of a combination of white and grey matter lesions detected on visual inspection of MR images was associated with IQ scores. For this analysis it was not further specified which grey matter structures were affected, or whether the periventricular lesions consisted of gliosis only or white matter reduction.

Statistical testing showed only very weak evidence for an association between the presence of a combination of white and grey matter lesions and IQ scores (see table 10.1, Mann-Whitney U test, Exact, p=0.1 for PIQ, and p=0.09 for VIQ).

10.2 Associations between epilepsy, findings on visual inspection of MR images, and Performance and Verbal IQ

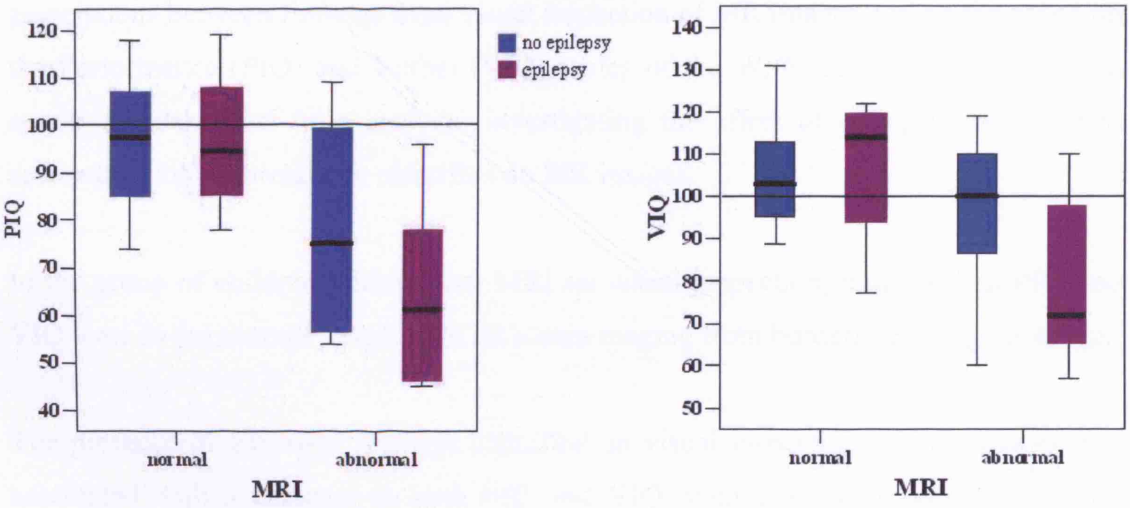
In chapter 6, section 6.3, it was shown that in the group with epilepsy PIQ was significantly lower than in the group without epilepsy, whereas there was only very weak evidence for a significant difference in VIQ between these two groups. Once neurological status had been controlled for, the non-significant associations between epilepsy and VIQ remained unchanged whereas the difference in PIQ was no longer significant.

The purpose of this section is to compare cognitive function between those with and those without epilepsy, taking into account findings from visual inspection of MR images. Four (22%) out of the 18 children with normal MRI, and 11 (42%) of the 26 children with abnormal findings on visual inspection of MR images had epilepsy. Figure 10.1a and figure 10.1b below show PIQ (figure 10.1a) and VIQ (figure 10.1b) for those with and those without epilepsy according to the MRI categories normal and abnormal MRI.

Within the category normal MRI, the median PIQ for those without epilepsy was 97.5 (min 74, max 118), and for those with epilepsy was 94.5 (min 78, max 119). The median VIQ for those without epilepsy was 103 (min 89, max 131), and for those with epilepsy 114 (min 77, max 122). Neither for PIQ nor for VIQ was there a significant

difference between the group with and the group without epilepsy (Mann-Whitney U test, Exact, p=0.9 for PIQ, p=0.6 for VIQ).

Within the category abnormal MRI, the median PIQ for those without epilepsy was 75 (min 54, max 109), and for those with epilepsy 61 (min 45, max 96). The median VIQ for the group without epilepsy was 100 (min 60, max 119), and for the group with epilepsy 72 (min 57, max 110). For both IQ scales statistical testing showed significant differences between the two groups (Mann-Whitney U test, Exact, p=0.04 for PIQ, p=0.03 for VIQ). The proportion of those with severe periventricular white matter reduction (as opposed to mild/moderate reduction) was greater in the group with epilepsy. In addition, in those with epilepsy, widespread subtle VBM-detected grey matter abnormalities (indicating more severe brain injury) were more frequent when compared to those without epilepsy (see chapter 9 for detailed discussion).



Figures 10.1a and 10.1b: Performance IQ (PIQ, figure a) and Verbal IQ (VIQ, figure b) on the WISC-R. Comparison between those with epilepsy and those without epilepsy in the MRI categories normal (n=18) and abnormal (n=26). In the group with normal MRI, 4 children had epilepsy and 14 children did not have epilepsy. In the group with abnormal MRI, 11 children had epilepsy and 15 children had no epilepsy. IQ scores are displayed on the Y-axis. Displayed is the median (thick solid black lines), minimum, maximum (indicated by the whiskers), and 25%-75% interquartile range (indicated by the box).The thin black horizontal line displays the mean/median of 100 for a normative population.

To further explore the effect of epilepsy in the context of brain abnormalities as indicated by visual inspection of MR images, a two by two factorial analysis of variance was performed, with PIQ and VIQ respectively as the dependent variables, and epilepsy (yes/no) and MRI (abnormal/normal) as independent variables. For both PIQ and VIQ, there was a significant difference for the two MRI categories (PIQ: $F(1, 40)=14.3$, $p=0.001$; VIQ: $F(1,40)=9.5$; $p=0.004$) but not for epilepsy (PIQ: $F(1,40)=1.2$; $p=0.3$; VIQ: $F(1,40)=1.4$; $p=0.2$). (It has to be noted that these additional analyses are not non-parametric tests. However, since the nature of these additional analyses was of explorative character, it was felt that it was appropriate to apply these tests in this context.)

10.3 Summary

In the sections above, findings were presented from univariate analyses investigating associations between findings from visual inspection of MR images and performance on the Performance (PIQ) and Verbal (VIQ) scales of the WISC-R. In section 10.2, the results are described from analyses investigating the effect of epilepsy on IQ when accounting for abnormalities identified on MR images.

In the group of children with normal MRI on visual inspection, both median PIQ and VIQ were in the average range, with IQ scores ranging from borderline to high average.

The presence of abnormal findings identified on visual inspection of MR images was associated with a decrease in both PIQ and VIQ, with the PIQ scores being more affected than the VIQ scores. Interestingly, the IQ profiles were similar in the group with normal MRI and the group with abnormal MRI. However, this pattern with VIQ being higher than PIQ, was more pronounced in those with abnormal MRI compared to those with normal MRI.

In the subgroup of those with visually identified abnormalities on MR images, there was a significant association between PIQ and presence of periventricular abnormalities, but no significant association between VIQ and periventricular white matter lesions. However, the severity of periventricular white matter reduction showed a significant

association with both PIQ and VIQ, and this remained once those with additional grey matter lesions on visual inspection of MR images were removed from the analysis.

The presence of grey matter abnormalities was associated with a decrease in both PIQ and VIQ. However, the majority of those with visible grey matter abnormalities had periventricular white matter lesions and thus is was difficult to examine in detail the effect of pure grey matter lesions on IQ scores. In addition, it was difficult to examine formally and/or in detail the effect of hippocampal abnormalities, cortical lesions, basal ganglia lesions, or thalamus lesions since in these subcategories, in the majority, lesions were seen in combination with other grey or white matter lesions and, in addition, in some of the these subcategories numbers were small.

Within the group of those with normal MRI findings, there was no significant difference in IQ scores between those with and those without epilepsy. Within the group of those with abnormal MRI findings, there was a significant difference between those with and those without epilepsy for both PIQ and VIQ. However, when an additional analysis was performed to examine the effect on IQ scores of both epilepsy and abnormalities on visual inspection of MR images, the effect of epilepsy on IQ scores was no longer significant, whereas the effect of abnormal findings on MR images remained for both IQ scales.

10.4 Associations of VBM-detected grey matter abnormalities with Performance IQ and Verbal IQ

The main question to be addressed in this section is whether the presence of VBM-detected grey matter abnormalities is associated with performance on the WISC-R. This analysis is performed first on all 40 datasets and then separately for those with visible abnormalities and those without visible abnormalities on MRI images.

Table 10.2 below shows associations of VBM-detected grey matter abnormalities with Performance IQ and Verbal IQ in the whole group of 40 children. For both PIQ and VIQ, the median IQ scores were lower in those with VBM-detected grey matter abnormalities, and this difference was more pronounced for PIQ. The statistical analysis

did not suggest that the difference in IQ scores between those with and those without VBM-detected grey matter abnormalities was significant (see table 10.2 below). However, when analysis were performed to test for associations between IQ scores and number of VBM-detected grey matter abnormalities (none, 1-2 focal, >=3 widespread; with widespread VBM abnormalities indicating widespread grey matter damage), there was a negative correlation between the number of VBM-detected abnormalities and scores on both IQ scales. PIQ was significantly lower when widespread abnormalities were present (Spearman's ρ=-0.4, p=0.03). Statistical testing did not, however, show a significant association between the number of VBM-detected grey matter abnormalities and VIQ (see table 10.2 below).

Table 10.2: Associations between VBM-detected grey matter abnormalities and IQ scores in the whole group (n=40)

VBM abnormalities (n=40 datasets)		**Performance IQ** *Median (min-max)*	*p-value*	**Verbal IQ** *Median (min-max)*	*p-value*
VBM grey matter abnormalities detected	n=23	82 *(45-109)*	**0.19***	99 *(60-119)*	**0.42***
No VBM grey matter abnormalities detected	n=17	96 *(54-118)*		103 *(68-131)*	
#Number of VBM-detected grey matter abnormalities					
0	n=17	96 *(54-118)*		103 *(68-131)*	
1-2 ("focal")	n=15	92 *(58-109)*	**ρ= -0.4;**	100 *(60-117)*	**ρ= -0.19;**
>=3 ("widespread")	n= 8	54 *(45-109)*	**p=0.03****	90.5 *(64-119)*	**p=0.25****

#VBM= voxel-based morphometry; classification of VBM-detected grey matter abnormalities (for details see chapter 8, section 8.2.6.): 0: no difference in grey matter density between the dataset of a preterm child and the datasets of the control group detected; 1-2 ("focal"): one or two peaks of differences in grey matter density between the dataset of a preterm child and the control datasets or multiple peaks within one cluster/confined structure detected; >=3 ("widespread"): three or more peaks that were not within one cluster/confined structure.

* Comparison between the group with and the group without VBM-detected grey matter abnormalities; Mann-Whitney U test, Exact.

** Spearman Rank test; ρ= Spearman's rho

Similarly to the exploratory analysis described in section 10.2 above, a two by three way analysis of variance was performed to explore the effect of epilepsy in the context of the number of VBM-detected grey matter abnormalities, with PIQ as the dependent variable and number of VBM-detected abnormalities (0, 1-2, >=3) and epilepsy (yes/no) as the independent variables was performed. There was a significant effect for the number of detected VBM abnormalities ($F(2,36)=4.8$, $p=0.01$) and weak evidence for an effect of epilepsy ($F(1,36)=3.6$; $p=0.07$). Since there was neither a significant association between VIQ and VBM abnormalities nor between epilepsy and VIQ, this exploratory analysis was not done for VIQ.

10.4.1 Subtle grey matter abnormalities detected by VBM analysis and associations with IQ scores – group with normal MRI on visual inspection

Seventeen (42%) of the 40 children included in the VBM analyses had normal MRI on visual inspection. In 8 of those 17 datasets, VBM detected subtle grey matter abnormalities. These VBM-detected abnormalities were focal in seven datasets (AS, MD, CS, SF, BA, SDaW, SSk) and widespread in one dataset (JRu). There was one child in this group of children with normal MRI and VBM-detected grey matter abnormalities who had epilepsy (SSk).

The location of the abnormalities were in the cortical grey matter in five datasets (n=3 frontal lobe, n=1 parietal lobe, n=1 temporal lobe), in the thalamus in two datasets, and in one dataset in both the cortical grey matter and the thalamus. In this latter dataset in which more widespread abnormalities were seen, they were located in the thalami, basal ganglia, and the temporal lobes bilaterally.

In both groups, those with and those without VBM-detected grey matter abnormalities, both PIQ and VIQ scores ranged from the upper end of the low average to the high average range, except in one child (JR; widespread VBM abnormalities), who had a PIQ in the borderline range. The one child with epilepsy had IQ scores in the average (PIQ) to high average (VIQ) range. For both IQ scales, the median IQ was higher in the group without VBM-detected grey matter abnormalities (PIQ: median 97, min 74, max 118; VIQ: median 111, min 77, max 131) compared to those with VBM-detected grey matter abnormalities (PIQ: median 93, min 76, max 109; VIQ: median 100.5, min 89, max 117). On statistical testing there was no evidence that these differences were significant (Mann-Whitney U test, Exact, p=0.49 for PIQ, p=0.84 for VIQ).

10.4.2 Subtle grey matter abnormalities detected by VBM analysis and associations with IQ scores – group with abnormal MRI on visual inspection

Twenty three (58%) of the 40 children who were included in the VBM analyses had abnormal findings on visual inspection of MR images. In 15 (65%) of these 23 datasets, VBM-detected grey matter abnormalities were seen. The VBM-detected grey matter

abnormalities were focal in eight datasets (BBe, DSk, NK, GO, TC, SH, BK, LB) and widespread in seven datasets (DHay, LO, CO, PD, WS, AM, LR). Four out of the eight children with focal VBM-detected abnormalities and four out of the seven children with widespread VBM-detected abnormalities had epilepsy.

In those with focal abnormalities, the abnormalities were located in the cortical grey matter in five cases (n=3 temporal lobe, n=1 parietal lobe, n=1 frontal lobe), in the cerebellum in two cases, and the hippocampus in one case. In those with widespread abnormalities, in all cases except one (PD), more than one cortical grey matter region was affected. In all datasets except one (AM), these were combined with basal ganglia/thalamus abnormalities. Additional cerebellar grey matter abnormalities were detected in two cases (PD, WS).

In both groups, those with and those without VBM-detected grey matter abnormalities, PIQ scores ranged from below borderline to average, and VIQ scores ranged from the upper end of below borderline to high average range. For the Performance Scales, the median PIQ was higher in those without VBM-detected grey matter abnormalities (median 95.5, min 54, max 101) compared to those with VBM-detected grey matter abnormalities (median 70, min 45, max 109). This difference was, however, not significant (Mann-Whitney U test, Exact, p=0.42). Median Verbal IQ was similar in the two groups (for those with VBM abnormalities: median 90, min 60, max 119; for those without VBM abnormalities: median 91.5, min 68, max 117; Mann-Whitney U test, Exact, p=0.92).

There was only very weak evidence for a correlation between the number (0, 1-2=focal, or >=3 =widespread) of VBM-detected grey matter abnormalities and PIQ (Spearman's, ρ= -0.36, p=0.09) and no evidence for a correlation with VIQ (Spearman's ρ= -0.08, p=0.71).

When only those who had only periventricular white matter abnormalities but no grey matter abnormalities on visual inspection of MR images were included in the analyses (n=5 without and n=8 with VBM-detected grey matter abnormalities), the patterns of associations between IQ scores with the presence of VBM-detected grey matter abnormalities remained similar (Mann-Whitney U test, Exact, p=0.47 for PIQ, p=0.94

for VIQ), i.e. non-significant. When exploring (the small number of cases remaining after exclusion of those with visible grey matter lesions limited this analysis to an exploratory analysis, which has to be kept in mind when interpreting the results) the associations between number of VBM-detected grey matter abnormalities and IQ scores, the previously seen, though very weak, effect on PIQ ($p=0.09$) was no longer seen although the correlation coefficient did not change substantially (for PIQ: Spearman's $\rho= -0.35$; $p=0.2$; for VIQ: $\rho= -0.16$, $p=0.6$).

10.4.3 Summary

In the sections above, associations between VBM-detected grey matter abnormalities and IQ scores were examined. For the whole group, no significant associations between either PIQ or VIQ and the presence of VBM-detected grey matter abnormalities were identified. When investigating associations between the number of detected VBM abnormalities (a surrogate measure for the extent of subtle grey matter damage) and IQ scores, statistical testing suggested a significant association between the number of VBM abnormalities (categorised as none, focal, widespread) and PIQ. There was no evidence for an association between the number of VBM-detected grey matter abnormalities and VIQ.

In the whole group, an additional analysis was performed exploring the effect of epilepsy in the context of the number of VBM-detected brain abnormalities on PIQ. It showed only weak evidence for an effect of epilepsy but a strong effect for "number of VBM-detected grey matter abnormalities", with lower PIQ when widespread grey matter abnormalities were present.

Within the subgroup of those with normal MRI on visual inspection, both the median PIQ and VIQ scores in the group without VBM abnormalities were higher than in the group with VBM-detected brain abnormalities. However, on statistical testing these differences were not significant.

Within the subgroup of those with abnormal MRI on visual inspection, median PIQ was higher in those without VBM abnormalities but this difference was not significant.

Verbal IQ was similar in the group without VBM abnormalities compared to the group with VBM abnormalities. When examining associations between the number of VBM-detected grey matter abnormalities and PIQ, there was only very weak evidence for an association with the number of VBM-detected grey matter abnormalities, and no evidence for a significant association with VIQ.

A formal analysis of associations between IQ scores and location of VBM-detected grey matter abnormalities was not attempted since the numbers in the categories for location of VBM abnormalities were very small.

10.5 Regression analyses

The aim of this section is to present results from linear regression analyses that were performed to investigate whether information obtained from neuroimaging improves prediction of overall cognitive outcome further to those clinical variables that have been identified as clinically useful predictors for overall cognitive outcome (see chapter 6). Furthermore, it was examined whether VBM-detected grey matter abnormalities would improve prediction of cognitive outcome compared with findings obtained from visual analysis of MR images alone.

Data were included in the regression analyses from children (n=40) for whom data were available from all three visual inspection of MR images, results from VBM analysis and psychometric assessments,

In the regression analyses that are presented in the current chapter, clinical variables that have been identified by the previous statistical analyses (presented in chapter 6) as being predictors of overall cognitive function have been included. In these analyses, TOMI error scores (Test of Motor Impairment) and one of the principal component scores (PCA scores) derived from perinatal and neonatal variables (see chapter 6, section 6.4.3 for details), i.e. PCA_1, which is dominated by gestational age at birth, birth weight, and duration of oxygen supplementation, were identified as the best independent clinical predictors of Performance IQ (PIQ). For Verbal IQ (VIQ), the best clinical predictor identified by the regression analyses was PCA_1. In addition to these

variables, in the analyses described in the current chapter, epilepsy was included as a variable of interest.

Based on the results obtained from the univariate analyses presented in previous sections of this chapter, the following imaging variables were examined in the regression analyses: normal/abnormal MRI on visual inspection, presence of periventricular white matter abnormalities, degree of white matter reduction, presence of grey matter abnormalities, hippocampal abnormalities detected on visual inspection of MR images, and the number of VBM-detected grey matter abnormalities (0 = none; 1-2 = focal; >=3 = widespread). The possible effects of cortical grey matter abnormalities and basal ganglia/thalamus abnormalities identified on visual inspection of MR images were explored but not formally examined.

Variables were entered one at a time and the contribution of each variable to the explanation of the variance of the outcome variable (PIQ or VIQ respectively) was assessed at each step.

10.5.1 Results from the regression analyses

10.5.1.1 Performance IQ

The results obtained from the examination of clinical variables (see chapter 6, sections 6.4.3.1.1 and 6.4.3.1.2) were used as a base model and imaging variables were added one at a time. As described in chapter 6, section 6.4.3.1.1, there was weak evidence (p=0.06) that epilepsy was independently related to PIQ; this effect however, disappeared once TOMI scores had been entered into the model. Thus, the effect of epilepsy, which was of overall interest in this study, was only examined in an exploratory way in the analyses performed here.

When the variable normal/abnormal MRI on visual inspection was added to the model that contained TOMI score and PCA_1, there was weak evidence that the presence of any abnormality on visual inspection contributed to the prediction of PIQ (p=0.06).

With this variable in the model, the effect of PCA_1 was very weak (p=0.09), and the effect of TOMI score remained, although weaker (p=0.03).

It was explored whether there was an independent effect of epilepsy once the perinatal variables (represented by PCA_1) had been removed from the model. With the variables "normal/abnormal MRI on visual inspection", TOMI score, and epilepsy in the model, there was no longer very strong evidence for an independent effect of TOMI score (p=0.09); neither did epilepsy contribute to the explanation of PIQ (p=0.4). Only the variable normal/abnormal MRI on visual inspection contributed independently (p=0.02) to the prediction of PIQ in a significant way.

It was further explored which of the categories of abnormal MRI findings might be the best predictor for PIQ. To this end, the variables periventricular white matter abnormalities, degree of white matter reduction, grey matter abnormalities, hippocampal abnormalities detected on visual inspection of MR images, and, finally, the number of VBM-detected grey matter abnormalities (0, 1-2 focal, >=3 widespread) were examined one at a time. These analyses were preformed twice, once without and once with TOMI score in the model.

Without TOMI score in the model, the results suggested that both periventricular white matter abnormalities and grey matter abnormalities identified on visual inspection of MR images predicted PIQ, though the evidence was not very strong (p=0.05 for grey matter abnormalities, p=0.06 for periventricular white matter abnormalities). A similar pattern (p=0.03 for white matter reduction, p=0.09 for grey matter abnormalities) was observed when the severity of periventricular white matter damage was taken into account by entering the variable "degree of white matter reduction" (none, mild/moderate, severe) instead of presence or absence of periventricular white matter abnormalities. When TOMI score was added to this model, the independent effect of white matter abnormalities disappeared (p=0.7), whereas an effect remained for grey matter abnormalities (grey matter abnormalities p=0.04, TOMI score p=0.007), indicating that TOMI score and white matter reduction were correlated, with TOMI score having a greater effect on PIQ.

It was further explored whether hippocampal abnormalities identified on visual inspection of MR images contributed independently to the prediction of PIQ. Again, this was first explored with the variables for periventricular white matter abnormalities but without TOMI score in the model. For this model, there was evidence for both an independent association of hippocampal abnormalities with PIQ and an independent association with PIQ for the variables presence/absence of periventricular white matter lesions as well as degree of periventricular white matter reduction ($p=0.03$ for hippocampal abnormalities when entered together with presence/absence of periventricular white matter lesions, $p=0.04$; $p=0.05$ for hippocampal abnormalities when entered together with the variable degree of periventricular white matter reduction, $p=0.01$). When TOMI score was entered in the model, the effect of white matter abnormalities (both presence/absence of periventricular white matter abnormalities, $p=0.6$; and degree of white matter reduction, $p=0.2$) disappeared, whereas there was still evidence for hippocampal abnormalities as independent predictors of PIQ ($p=0.03$) as well as for TOMI score ($p=0.009$ when in the model with presence/absence of periventricular white matter abnormalities, $p=0.02$ when in the model with degree of white matter reduction). Neither cortical abnormalities nor abnormalities of basal ganglia or thalami had an effect on prediction of PIQ.

The variable number of VBM-detected grey matter abnormalities was added to the models that contained the white matter variables and the variable hippocampal abnormalities. For the model without TOMI score included, there was an independent effect of both hippocampal abnormalities (weak effect, $p=0.08$) and number of VBM-detected grey matter abnormalities ($p=0.02$) when entered together with the white matter variables. In this model, there was no evidence for an independent association of white matter variables with PIQ ($p=0.3$ for presence/absence of periventricular white matter abnormalities, $p=0.2$ for degree of white matter reduction). Similarly, with TOMI score in the model, both hippocampal abnormalities and VBM-detected grey matter abnormalities were independent predictors of PIQ. Table 10.3 below shows the results of the final model that retained the variables TOMI score, presence of hippocampal abnormalities identified on visual inspection of MR images, and number of VBM-detected grey matter abnormalities as the variables that contributed independently in a significant way to the prediction of PIQ.

Table 10.3: Results of the regression analysis with the PIQ as the dependent variable; final model

	B	SE	t-value	p - value	95% CI
Constant	84.4	8.1	10.5	0.00	68.0 - 100.7
TOMI*	-1.7	0.6	-2.6	0.01	-0.3 - -0.39
Number of VBM-detected grey matter abnormalities#	-7.9	3.8	-2.1	0.04	-15.7 - -0.23
Hippocampal abnormalities on visual inspection of MRI images	16.6	7.7	2.1	0.04	0.89 – 32.4

B= regression coefficient, SE=standard error, CI= confidence interval
* TOMI= Test of Motor Impairment, error score
# VBM= voxel-based morphometry; classification of VBM-detected grey matter abnormalities (for details see chapter 8, section 8.2.6.): 0: no difference in grey matter density between the dataset of a preterm child and the datasets of the control group detected; 1-2 ("focal"): one or two peaks of differences in grey matter density between the dataset of a preterm child and the control datasets, or multiple peaks within one cluster/confined structure detected; >=3 ("widespread"): three or more peaks that were not within one cluster/confined structure.

In summary, of the available clinical and imaging data, the best independent predictors for PIQ were the presence of hippocampal abnormalities on visual inspection of MR images, the number of VBM-detected grey matter abnormalities (none, focal, widespread), and TOMI error scores (measure of neuromotor impairment, see also chapter 6, section 6.4.3.1). Perinatal variables contained in PCA_1 (determined by gestational age at birth, birth weight, and duration of oxygen supplementation), that had been identified in the regression analyses examining purely clinical variables, were no longer independent predictors of PIQ once imaging variables had been added to the regression model. Interestingly, the periventricular white matter abnormalities, and within this category, the severity of periventricular white matter reduction, were only independent predictors for PIQ without TOMI score and without VBM-detected grey matter abnormalities in the model, suggesting that TOMI scores and periventricular white matter abnormalities are correlated.

10.5.1.2 Verbal IQ

As described in chapter 6 the variable PCA_1 had been identified as the only clinical variable that contributed independently in a significant way to prediction of VIQ. There was weak evidence (p=0.08) that epilepsy was independently related to VIQ; this effect, however, disappeared once TOMI score and PCA_1 had been added to the model. However, since the effect of epilepsy on cognitive outcome was of general interest in this study, it was also explored whether there was an independent effect of epilepsy.

When the variable normal/abnormal MRI on visual inspection was added to the model that contained PCA_1 and epilepsy, the effect of epilepsy disappeared (p=0.12), with PCA_1 (p=0.02) and the variable normal/abnormal MRI on visual inspection (p=0.04) remaining significant independent predictors of VIQ.

It was further explored whether specific abnormal MRI findings (rather than only the variable normal/abnormal MRI) might be significant independent predictors for VIQ. To this end, the variables periventricular white matter abnormalities, degree of white matter reduction, grey matter abnormalities on visual inspection of MRI, and hippocampal abnormalities detected on visual inspection of MR images, and finally number of VBM-detected grey matter abnormalities (0, 1-2 focal, >=3 widespread) were examined one at a time.

Neither the variable presence/absence of periventricular white matter abnormalities nor the degree of white matter reduction were identified as independently associated with VIQ when added to the model that contained PCA_1 (p=0.2 for presence/absence of periventricular white matter abnormalities, p=0.3 for degree of white matter reduction; p=0.01 for PCA_1).

When the variable presence/absence of grey matter abnormalities on visual inspection was entered in the model together with PCA_1, the analysis suggested that both variables were independent significant predictors of VIQ (p=0.02 for PCA_1, p=0.04 for grey matter abnormalities). For the subcategories of grey matter abnormalities, neither cortical abnormalities nor basal ganglia/thalamus abnormalities contributed to the prediction of VIQ. There was weak evidence that the presence of hippocampal

abnormalities on visual inspection of MR images predicted VIQ (p=0.07) together with PCA_1 (p=0.01) but this effect was small and only seen when the variable grey matter abnormalities was not entered into the model, suggesting that within the variable grey matter abnormalities there was, in addition to the hippocampal abnormalities, a contribution of cortical lesions and basal ganglia/thalamus lesions. There was no evidence that the variable number of VBM-detected grey matter abnormalities was independently predictive of VIQ (p=0.8) with the other imaging variables in the model. Table 10.4 below shows the results of the final model that retained the variables PCA_1 and grey matter abnormalities on visual inspection of MR images as the variables that contributed independently in a significant way to the prediction of VIQ.

Table 10.4: Results of the regression analysis with VIQ as the dependent variable; final model

	B	**SE**	**t-value**	**p – value**	**95% CI**
Constant	87.0	5.0	17.3	0.00	76.7 – 97.0
PCA_1*	3.6	1.6	2.3	0.02	0.4 – 6.9
Grey matter abnormalities on visual inspection#	12.7	6.1	2.0	0.04	0.3- 25.1

B= regression coefficient, SE=standard error, CI= confidence interval
* PCA scores = derived scores calculated from: gestational age, birth weight, APGAR5, PDA, duration of oxygen supplementation, ultrasound category; PCA_1 determined by birth weight, gestational age and by duration of oxygen supplementation.
# Any grey matter abnormality detected on visual inspection of MR images; weak (non-significant) effect of hippocampal abnormalities on visual inspection only when not in model together with the variable grey matter abnormalities

In summary, the best predictors for VIQ were PCA_1 (determined by gestational age at birth, birth weight, and duration of oxygen supplementation) and grey matter abnormalities on visual inspection of MRI (with weak evidence for an effect of hippocampal abnormalities when not in the model together with the variable grey matter abnormalities) being independently predictive of VIQ. None of the other individual MRI categories (periventricular white matter abnormalities, degree of white matter reduction, cortical grey matter, basal ganglia or thalamus abnormalities) contributed independently to prediction of VIQ. VBM-detected grey matter abnormalities did not

improve prediction for VIQ over the information obtained from visual inspection of MR images.

10.5.2 Summary of the results from the regression analyses

The regression analyses indicated that for both IQ scales findings from MR imaging provided useful prognostic information in addition to the previously identified clinical variables. For PIQ, the best predictors from neuroimaging were the number of VBM-detected grey matter abnormalities (with widespread abnormalities predicting lower PIQ) and hippocampal abnormalities identified on visual inspection of MR images. For VIQ, the best predictor from neuroimaging was grey matter abnormalities identified on visual analysis of MR images. For neither PIQ nor VIQ were periventricular white matter abnormalities independent significant predictors.

For VIQ, in addition to grey matter abnormalities on visual inspection of MR images, the clinical variable PCA_1 (which reflects gestational age at birth, birth weight, and duration of oxygen supplementation) remained a strong independent predictor. For PIQ, however, PCA_1 was no longer an independent predictor once imaging variables were taken into account. However, TOMI score, a measure of neuromotor function and/or impairment remained an independent predictor together with the imaging variables described above. Epilepsy was not identified as an independent predictor for either PIQ or VIQ.

10.6 Discussion

The main objective of the analyses presented in this chapter was to investigate how cognitive function as indicated by performance on the WISC-R and brain pathology as indicated by findings from qualitative analysis (i.e. visual inspection of MR images) and quantitative analysis (i.e. VBM analysis of grey matter segments) of MR data are associated in the group of preterm children examined in this study. Second, results from regression analyses were presented that examined which of the available data provided clinically useful prognostic information for cognitive outcome, and, in particular, whether information obtained from neuroimaging improves prediction of overall cognitive outcome in addition to clinical variables. Furthermore, findings from analyses exploring the effect of epilepsy on cognitive outcome were described.

For a number of children either no information from WISC-R assessments was available or, the severity of their motor impairments did not allow them to complete the required number of subtests on the IQ scales needed for computation of IQ scores. Thus, for the analyses examining associations between findings from visual inspection of MR images and IQ scores, 44 out of the available 54 MR data sets were used. Similarly, for the analyses investigating associations between IQ scores and VBM-detected grey matter abnormalities, and for the regression analyses, some data sets had to be excluded because pre-processing for VBM gave poor results. The majority of those excluded had epilepsy and/or large periventricular white matter lesions on visual inspection of MR images (for details see introduction to this chapter and chapter 6, section 6.1). It has to be kept in mind when interpreting the findings of the analyses presented in this chapter, that the exclusion of those data might have introduced a bias.

For the analyses performed in this chapter, similar issues to those discussed in chapter 9, section 9.5.7, apply, in particular, with regard to the aims of the regression analyses, sample size in some of the univariate analyses, and some technical and methodological issues concerning the VBM analyses.

10.6.1 Neuroimaging findings and associations with IQ scores

The analyses described in this chapter showed that an abnormal MRI scan on visual inspection was significantly associated with a decrease in both PIQ and VIQ, irrespectively of the presence or absence of epilepsy. In the group with an abnormality on MRI, the variation of IQ scores was considerably larger than in the group of those with normal MRI. When examining in more detail the associations between the specific categories of brain abnormalities and IQ scores, the analyses suggested that the presence of periventricular white matter abnormalities (i.e. either gliosis only or white matter reduction with or without gliosis) was significantly associated with a decrease in PIQ but not in VIQ. Interestingly, for the group of children with gliosis only (and no reduction of periventricular white matter) median IQ scores were in the average range for both VIQ and PIQ. When taking the degree of white matter reduction into account, there was a significant negative correlation with both PIQ and VIQ scores. The presence of grey matter lesions on visual inspection of MR images was significantly associated with a decrease in both IQ scores. The analyses further indicated that hippocampal abnormalities were significantly associated with both PIQ and VIQ. The analyses performed for the whole group to investigate associations between subtle grey matter abnormalities detected by VBM, showed no significant association between the presence of VBM-detected abnormalities and IQ scores. There was, however, a significant correlation between the number of VBM-detected grey matter abnormalities and PIQ, suggesting that in those with low PIQ widespread brain injury is present including both grey matter (as indicated by VBM analysis) and white matter (as indicated by visual inspection of MR images). This interpretation is supported by the finding that the decrease in PIQ was most pronounced in those with severe degrees of periventricular lesions on visual inspection of MRI, and that in this study a strong correlation was found between the number of VBM-detected grey matter abnormalities and the degree of white matter reduction (see chapter 8).

The main results from the analyses investigating findings on visual analysis of MRI images with overall cognitive outcome are consistent with previous studies. For example, Olsen et al, (1998), in a population based study of 42 children born with a birth weight <1750 g, examined associations between performance on the WISC-R and subtests of the NEPSY with findings on visual inspection of MR images. Half of the

children had normal neurological findings, 28% had minor neurological signs, 10% had a diagnosis of cerebral palsy. Thirty-two percent of the sample had "PVL" (in their study defined as abnormally increased signal on T2 weighted images and/ or a reduced amount of periventricular white matter, and compensatory focal ventricular enlargement adjacent to the regions of abnormal signal intensity). Similarly to the current study, differences (even after excluding those with a diagnosis of CP) between the preterm children and a control group of term born children in performance on the WISC-R were most pronounced for PIQ, in particular, for the subtests that assess spatial and visuo-perceptual abilities (which fits with the location of PVL in the parieto-occipital regions in the majority of the cases in both the current study and the study by Olsen et al, 1998). Similar findings were reported by Skranes et al (1998), who examined, at the age of 6 years, a group of children with birth weight <1500g. They found that those with white matter abnormalities in the centrum semiovale and central occipital white matter had low scores on performance subtests. In the study by Olsen et al (1998), children with PVL scored significantly lower on both IQ scales than the control children. However, and this is not consistent with the findings from the current study and some other previous studies, within the preterm group, preterm children with and without PVL diagnosed on MR images had similar IQ scores. The authors conclude that in those without PVL and impaired performance on testing, subtle brain abnormalities that are not detectable by visual inspection of MR images might have been present and that these abnormalities are likely to explain the poor performance of those without a diagnosis of PVL.

Krägeloh-Mann et al (1999), in a study investigating 40 children (born between 27-34 weeks of gestation) at a mean age of 6.2 years found lower IQ scores in the preterm group compared to a control group of term born children. The severity of periventricular white matter reduction was associated with Full Scale IQ when taking the whole group into account. However, when only those children with an FSIQ above 70 were considered, no significant differences in IQ scores (VIQ and PIQ) were found between those with normal MRI and those with abnormal MRI. The only significant difference between the groups was in the frequency of the occurrence of attention deficit hyperactivity disorder (assessed with the Connors Scales). Since all children with an FSIQ <70 had bilateral extensive white matter lesions or cerebellar atrophy, and no child with a unilateral lesion or mild periventricular abnormalities had an FSIQ <70, the

authors concluded that unilateral lesions and mild bilateral lesions can, in contrast to severe bilateral lesions, be compensated for. The finding in the current study that those with mild white matter reduction or gliosis only had a better cognitive outcome than those with more severe white matter reduction, is consistent with the findings in the study by Krägeloh-Mann et al (1999). In the current study, it was not possible to formally examine the effect of unilateral versus bilateral brain lesions. Only three children had unilateral severe white matter reduction; two of those children had IQ scores in the average or low average range and one below average.

Some previous studies that examine associations between findings obtained from visual assessment of MR images with cognitive functioning of preterm children at late childhood/adolescence find, in contrast to the current study, no significant associations between abnormalities on MR images and outcome. For example, Cooke and Abernethy (1999), investigated a group of children (n=87) born between 24 -35 (mean 28.6) weeks of gestation, at the age of 12-13 years. Abnormal MRI, consisting of white matter abnormalities ranging from ventricular enlargement to porencephaly, was seen in 42.5% of this group. No significant associations between FSIQ and MRI abnormalities were found. However, in their study (Cooke and Abernethy, 1999), PIQ and VIQ were not considered separately and the degree of white matter reduction was not graded, making a direct comparison with the current study difficult. Stewart et al (1999) reported a high proportion (40/72) of abnormal findings on MRI (a spectrum of white matter lesions ranging from ventricular dilatation to cystic lesions) at the age of 14 years in a group of preterm children born <33 weeks of gestation. In their study, a range of psychometric tests for assessment of cognitive and behavioural function was administered, and the only significant association with MRI findings was seen for the Rutter Behavioural Scale. No differences between those with and without MRI abnormalities were found for reading and spelling abilities, or tests of visuo-motor integration. However, IQ scores were not examined and, similarly to the study by Cooke and Abernethy (1999), the MRI abnormalities were assessed according to a slightly different system; thus comparison with the current study is limited. Rushe et al (2001) conducted a further study based on the cohort and imaging data of the study carried out by Stewart et al (1999), in which (in the whole group and also when those with neurological impairments, i.e. neuromotor, visual and hearing impairments, were excluded), the only difference between those with and those without MRI abnormalities was found for

verbal fluency. For comparison with the current study, limitations apply as for the studies by Cooke and Abernethy (1999) and Stewart et al (1999).

Interestingly, in the study by Krägeloh-Mann et al (1999), in both preterm groups (those with and those without abnormalities on MRI), and irrespectively of neurological status (13 out of the 29 children in this study had a diagnosis of CP), PIQ was similar to or even higher than VIQ, which is not consistent with most existing studies that include children with CP, including the present study, in which PIQ was lower than VIQ (see chapter 6, section 6.3.1.3). For example, Fedrizzi et al (1993) examined a group of preterm children (born < 37 weeks GA) with "spastic diplegia" and periventricular lesions on MRI at the ages 6-14 years with the WPPSI/WISC-R. They found significant differences between VIQ and PIQ, with PIQ being lower than VIQ. Furthermore, their study showed that FSIQ and PIQ were significantly related to the severity of periventricular white matter reduction, involvement of the optic radiation, and thinning of the corpus callosum. They found no associations between any of the MRI abnormalities that were assessed (ventricular enlargement, white matter reduction, white matter hyperintensity on T2 weighted images, optic radiation involvement, thinning of the corpus callosum) and VIQ. A possible explanation for the differences seen in the IQ profile in the current study when compared with the study by Krägeloh-Mann et al (1999) might be that in the study by Krägeloh-Mann et al, data from psychometric assessments for only 4 out of the 13 children with CP were available, which might have resulted in an underestimation of impaired performance on the PIQ scale in their sample.

Abernethy, Cooke and Foulder-Hughes (2004), in a study that included qualitative and quantitative (volumetry) analysis of MR data, reported on a large group of preterm children without CP (n= 105; born < 32 weeks gestational age, examined at a mean age of 7.4 years with WISC-III and Movement ABC). Brain abnormalities were seen in 18.5% of the group. In their study, periventricular leukomalacia was defined as the triad of abnormally high signal in the periventricular white matter on T2 weighted images, loss of periventricular white matter, and compensatory ventricular enlargement adjacent to the regions of abnormal signal intensity. Quantitative assessment of MRI data included measurement of volumes of the caudate nuclei and the hippocampi. When all children were included in the analysis (i.e. those with and those without brain

abnormalities), lesions identified on visual inspection of MRI were associated with all three IQ scores and, in addition, significant correlations between volume of the caudate and IQ scores were seen. These correlations remained once cases with abnormal MRI had been excluded. However, when controlling for total brain volume, only PIQ was significantly correlated with caudate volumes. Interestingly, no associations between IQ scores and hippocampal volumes were found, which is in contrast to previous research (see discussion further below).

In the current study, thinning of the corpus callosum was associated with decrease in both PIQ and VIQ. Thinning of the corpus callosum in all children except one was associated with white matter abnormalities. Therefore it was not possible to investigate associations between IQ scores and corpus callosum thinning in the absence of other white matter abnormalities. Nosarti et al (2004), in a study on adolescents born preterm, found evidence for selective thinning of the corpus callosum and, in males only, an association of thinning of the mid-posterior portion of the corpus callosum with performance on a verbal fluency task and also with VIQ.

In the current study, grey matter abnormalities on MR images were significantly associated with both PIQ and VIQ decrease. In the majority of cases, the grey matter abnormalities were seen together with periventricular white matter abnormalities, which makes it difficult in univariate analyses to examine a possible direct effect of grey matter abnormalities on cognitive function. In addition, the frequency of cortical lesions as well as lesions in the basal ganglia and thalami was low, so that formal statistical testing was not possible. Four children with large cortical lesions (MCA infarct, schizencephaly) were not included in the analyses since they were unable to complete the required number of tests needed to calculate IQ scores. The exclusion of these children from the analysis might introduce a bias towards underestimation of a negative effect of large cortical lesions on overall cognitive function. Isolated abnormalities in basal ganglia and the thalami were seen in only one child; in the other three children abnormalities in these structures were associated with periventricular white matter lesions. This is consistent with previous studies (Yokochi, 1997; Krägeloh-Mann et al, 1999), in which thalamic lesions were seen in association with white matter reduction in the periventricular areas, and this was associated with very poor overall cognitive and visual function and poor motor function compared to those with white matter lesions but

no thalamic abnormalities. In the current study, too, abnormalities in the basal ganglia were seen in combination with periventricular white matter lesions, except in one child. IQ scores in these children were in the average range. In the above mentioned study by Abernethy, Cooke and Foulder-Hughes (2004), in a group of preterm children without major neuromotor impairment (but nevertheless with abnormal findings on MR images in 18.5 % of the study group), significant associations of volume of the left and right caudate with decrease in FSIQ were found, and this remained after excluding those with visible lesions on MRI. After controlling for brain volume, the associations persisted for PIQ only. Considering the central and complex role of the thalami and the basal ganglia with regard to projections to and from the cortex, it is not surprising that lesions, either primary or secondary, lead to impairments of various aspects of cognitive and motor function. As mentioned above, in the current study, the frequency of lesions in these structures was too low for a detailed and formal examination of possible effects on long term outcome.

In the current study, hippocampal abnormalities, (which, in the majority of the cases consisted of small hippocampi either unilaterally or bilaterally) on visual inspection of MR images were significantly associated with both VIQ and PIQ, with PIQ being more affected than VIQ. Associations between abnormalities in the hippocampi (hippocampal volumes assessed quantitatively) have been reported in previous studies for both adults and children. For example, Schumann et al (2007), in a study that included healthy 8-18 year old boys examined with an abbreviated version of the Wechsler Intelligence Scales and MRI, found that hippocampal volumes correlated positively with VIQ. The hippocampus is involved in learning of factual information, which is assessed in some subtests of the VIQ scale. In the study by Schumann et al (2007) no associations between hippocampal volume and PIQ were found. However, there are several studies that have found such an association in both typical and atypical populations (e.g. Andreasen et al, 1993; Abernethy, Cooke and Foulder-Hughes, 2004).

Given the role and importance of the hippocampus in learning and memory and in particular for spatial processing, it is likely that the pronounced decrease in PIQ in those in whom hippocampal abnormalities were detected in the current study is related to the spatial aspects of the PIQ subtest. Findings from previous studies using quantitative methods of MR analysis (e.g. Abernethy, Palaniappan and Cooke, 2002) support this

interpretation, although in some studies, including studies that investigated typical adult populations, PIQ was related to left hippocampal abnormalities only (Andreasen et al, 1993; Abernethy, Palaniappan and Cooke, 2002) whereas a reduction in VIQ was related to bilateral hippocampal abnormalities (Andreasen et al, 1993). In the current study, hippocampal abnormalities were seen bilaterally in the majority of the cases. It has to be kept in mind that in most cases in which hippocampal abnormalities were seen, periventricular white matter changes (mainly located in the parieto-occipital region) were present. As already indicated further above, parieto-occipital periventricular white matter reduction identified on visual inspection on MR images has been shown in previous studies to be associated with difficulties in visuo-spatial and visuo-perceptual function (Fedrizzi et al, 1996; Olsen et al, 1998), which plays a role in PIQ subtests. When those with both white matter lesions and hippocampal abnormalities were compared to those with white matter abnormalities but no hippocampal abnormalities, the IQ scores were not significantly different. One could speculate that periventricular white matter abnormalities in the population of the current study had a larger effect on IQ scores than hippocampal abnormalities identified on visual inspection of MR images. However, it is possible that in some cases, in particular those without white matter lesions, subtle hippocampal abnormalities are present that were not detected by either visual inspection or the VBM grey matter analysis (for the VBM analyses MR data were smoothed to 12 mm, which may result in failure to detect hippocampal abnormalities (for a detailed discussion, see chapter 8, section 8.4.4.2). Interestingly, most previous studies that investigate findings from visual analysis of MR images and associations with outcome in preterm children do not specifically include scoring of hippocampal abnormalities into their scoring systems but focus on assessment of white matter abnormalities.

In addition to visual inspection of MR images, VBM analysis was carried out to detect subtle grey matter abnormalities, and analyses were performed to examine associations between the VBM findings and cognitive function. A significant negative correlation was found between the number of VBM-detected grey matter abnormalities and PIQ only. Nosarti et al (2008), using VBM to examine grey and white matter distribution in a group of 218 preterm adolescents and 128 controls, found widespread areas of decreased and increased grey and white matter, with the greatest white and grey matter alterations seen in those with evidence of periventricular haemorrhage and ventricular

dilatation on neonatal ultrasound. The children in this study (Nosarti et al, 2008) underwent a large battery of psychometric tests and cognitive impairment was defined as performance of >= 1 standard deviation below the mean of the control group scores. They found that decreased grey matter and decreased white matter in several brain regions (grey matter in the parietal lobe, the frontal lobe and cingulate gyrus, white matter in the brainstem, temporal lobe) were predictive of cognitive outcome (language and executive function scores), irrespectively of being born preterm or term. Two regions in which increased density compared to controls was found were predictive of cognitive outcome (grey matter in the temporal lobe and white matter in the cingulate gyrus). The findings in the current study, i.e. that widespread VBM-detected abnormalities were seen mainly in those with periventricular white matter lesions and, furthermore, that widespread grey matter abnormalities are associated with cognitive outcome, are consistent with those of the study by Nosarti et al (2008). However, since different psychometric test batteries were used comparison of the findings with regard to specific measures of cognitive function and their associations with the detected brain abnormalities remains limited.

Isaacs et al (2001, 2003, 2004), in a series of studies in preterm children/adolescents without neuromotor impairment, examined associations between brain abnormalities and overall cognitive function and/or specific cognitive deficits. In their studies, focal brain abnormalities were detected, consisting of decrease in grey matter density in the temporal lobe (associated with visuo-spatial processing deficit; Isaacs et al 2003), the parietal lobe (associated with calculation difficulties; Isaacs et al, 2001), both the parietal and temporal lobe (associated with absolute IQ scores; Isaacs et al, 2004) and abnormalities in the frontal, temporal, and occipital lobes (associated with decline over time in VIQ and PIQ respectively; Isaacs et al, 2004). The majority of the children in these studies had normal MRI on visual inspection (only few had minor/moderate brain abnormalities on visual inspection of the MR images, which included small corpus callosum, delay of myelination, mild "PVL", or small hippocampi). Of particular interest in the context of the current study is the study investigating associations with IQ scores (Isaacs et al, 2004), since similar psychometric variables were examined (PIQ and VIQ) and VBM was used for image analysis. In the current study, in 7 of the 17 datasets that were judged as normal on visual inspection, VBM analysis detected subtle grey matter abnormalities, which, in all but one dataset, were focal. The focal VBM-

detected grey matter abnormalities were located in the frontal, temporal and parietal lobes and in the thalami, i.e. areas that include those identified in the Isaacs et al (2004) and in other previous studies (e.g. Nosarti et al, 2008) investigating cognitive outcome and imaging findings in preterm children. However, in the current study, no pattern of associations between the location of these focal abnormalities and IQ scores emerged, which might partly be explained by the very small number of datasets in this subgroup.

10.6.2 Neuroimaging findings, epilepsy and cognitive function

In chapter 6, it was shown that there were differences in cognitive outcome between the group with epilepsy and the group without epilepsy, with statistical testing providing strong evidence for children with epilepsy performing poorer on PIQ tests and weak evidence for poorer performance on VIQ scales. The analyses presented in the current chapter, which take neuroimaging findings into account, showed that within the group with normal MRI findings, there were no significant differences in IQ scores between those with and those without epilepsy. In the group with abnormal MRI, for both PIQ and VIQ significant differences between those with and those without epilepsy were seen. In the group with epilepsy, white matter reduction was more severe and VBM-detected grey matter abnormalities were more widespread than in those without epilepsy, suggesting that the difference in IQ scores might be a consequence of more severe brain injury. This interpretation is supported by the finding that, when the effect of epilepsy on performance on IQ testing was examined in analyses that take brain abnormalities into account, the effect of epilepsy on PIQ and VIQ was no longer significant.

Although there is little information available specifically on preterm children with epilepsy, there are numerous studies that show that childhood epilepsy can be associated with a spectrum of cognitive and behavioural problems (for detailed discussion, see chapter 6, section 6.5.4). However, only few studies have investigated the relationships between findings on qualitative analysis of MR data, cognitive function and epilepsy. Furthermore there are no previous studies that focus entirely on preterm children and use both visual analysis of MR images and morphometric MR analysis for investigation of these relationships in such a population. Most previous research has been focused on

children with hemiplegia or bilateral spastic leg dominated CP ("diplegia") as a consequence of early acquired brain lesions. In most of these studies, the brain lesions are heterogeneous in terms of aetiology and timing of lesion, include both preterm and term born children, and often use no scoring system for qualitative analysis of MR images that take lesion extent and location into account, and furthermore do not include morphometric analysis of imaging data, which limits comparison with the current study. Nevertheless, such studies (e.g. Levine et al, 1987; Sussova, Seidl and Farber, 1990; Vargha-Khadem et al, 1994; Isaacs et al, 1996; Muter, Taylor and Vargha-Khadem, 1997) provide important information for discussion of relationships between early acquired brain lesions, cognitive function and the effect of seizures on cognitive outcome.

Isaacs et al (1996) investigated effects of early brain lesions (sustained prenatally or perinatally) on IQ (measured on the Wechsler Scale), manual abilities and dichotic listening in a group of 84 children with unilateral lesions and an IQ >70. Twenty seven of the children had a seizure disorder. IQ scores in those without seizures were not significantly different from controls whereas IQ scores in those with seizures were significantly lower than in controls and those without seizures. Furthermore, a history of seizures was significantly related to manual measures (slowing of tapping, reduced force) and a shift in handedness. Within the group with seizures, both VIQ and PIQ showed a substantial and equivalent reduction irrespectively of the side of lesion. The authors suggested that one possible explanation for their findings was that the presence of epileptiform activity or the drugs administered for treatment of seizures interfered with the brain tissue's potential for re-organisation of vulnerable functions in the already damaged hemisphere, whether left or right. They also suggested an alternative explanation, i.e. that seizures or their treatment interfered with normal function in the undamaged hemisphere. In contrast to the current study, in the study by Isaacs et al (1996) the aetiology of the brain lesions was heterogeneous, was not limited to inclusion of preterm infants, and all children were on anticonvulsant drugs. The effect of the severity (extent) of the brain lesions was not taken into account and there was no information on severity of motor impairment and how this differed between the groups. Only a small number of subjects had MRI brain scans or CT of the brain. An alternative explanation for the finding that in the seizure group IQ scores were significantly lower than in the group without seizures, would be that the presence of seizures reflect the

severity of the brain injury, which could be expected to have an effect on performance on many aspects of cognitive functioning. This explanation is consistent with results of a study in hemiplegic children that suggested that impairment on psychological measures is related to lesion size rather than presence of epilepsy (Levine et al, 1987). This interpretation would also be consistent with findings from the current study, i.e. that in those in whom the most severe degree of white matter reduction and widespread VBM-detected grey matter abnormalities were seen, a combination of severe motor impairment, impairment of overall cognitive function and epilepsy was more frequent than in those with less severe white matter abnormalities and/or focal VBM-detected grey matter abnormalities. An exploratory analysis that examined the effect of epilepsy on IQ scores indicated that there was no significant independent effect of epilepsy once such brain abnormalities were accounted for. Further support for the interpretation that the brain abnormalities rather than the epilepsy have a main effect on overall cognitive outcome is provided by the finding that there were no significant differences in IQ scores between those with and without epilepsy in the subgroup with normal MRI. Other studies have shown contradictory results, i.e. they suggested that the presence of seizures, not the extent of brain lesions, was the critical variable (Sussova, Seidl and Farber, 1990; Vargha-Khadem et al, 1994). Muter, Taylor and Vargha-Khadem (1997), based on a study that investigated 30 young children with hemiplegia and assumed unilateral brain injury acquired prenatally or perinatally, suggested that a unilateral injury to either hemisphere results in a selective decrease of PIQ, whereas a decrement in all IQ scores is a consequence of interference caused by seizures and/or medication. In the current study, the IQ profile was similar in those with and those without epilepsy with the PIQ being lower than VIQ. However, within the group with abnormal MRI on visual inspection, in the subgroup without epilepsy median VIQ was in the average range whereas PIQ was decreased. In contrast, in the group with epilepsy, both VIQ and PIQ were decreased. These findings appear consistent with those from the study by Muter, Taylor and Vargha-Khadem (1997) described above. It has to be kept in mind though that in the current study, in the group with seizures, the extent of periventricular white matter reduction was greater and widespread VBM-detected grey matter abnormalities were more frequent than in the group without epilepsy. Furthermore, brain lesions were bilateral in the majority of the cases. In the study by Muter, Taylor and Vargha-Khadem (1997), no MRI investigations were available, and for only 24 out of the 38 children included results from CT or ultrasound investigations were available.

No standardised scoring system of brain abnormalities or quantitative analysis of neuroradiological data was performed. Thus, it is possible that bilateral lesions had been missed and that the extent of lesions could not accurately be judged. Therefore it remains difficult to disentangle the effect of brain lesions and the effect of seizures on cognitive outcome in their study.

In the current study, the significant difference in IQ scores between those with and those without epilepsy was seen only in the subgroup of those with abnormal findings on MR images. Within the group with normal findings on visual inspection of MR images, no significant difference for either PIQ or VIQ was seen. In the subgroup with abnormal findings on MR images, a significant difference for both IQ scales was found. In the epilepsy group the degree of periventricular white matter reduction was more frequently severe compared to the group without epilepsy and when brain lesions were taken into account in exploratory tests, the effect of epilepsy on IQ scores disappeared and the effect of lesions identified on visual inspection of MR images remained. When a similar analysis was performed controlling for grey matter abnormalities detected by VBM analysis, there was only very weak evidence for epilepsy being associated with PIQ. Thus the overall results performed for investigation of associations between imaging findings, cognitive function and epilepsy, together with the findings presented in chapter 6, suggest that, in this study population, epilepsy can mainly be regarded as a marker for the severity of the brain injury, together with cognitive impairment, abnormal neuromotor status (CP) and impairment of motor function (as indicated by the TOMI scores).

10.6.3 Regression analyses

One of the main questions investigated in this chapter was whether information obtained from neuroimaging improves prediction of overall cognitive outcome further to those variables that have been identified in the analyses described in chapter 6 as clinically useful predictors for overall cognitive outcome in this group of preterm children.

Similarly to the regression analyses described in chapter 6 and chapter 9, it has to be kept in mind that these analyses were not performed with the aim of determining causation or quantify risk but rather to identify those variables that provide clinically useful prognostic information.

The results from the regression analyses suggest that for both PIQ and VIQ, neuroimaging variables, from both visual inspection and VBM analysis, add important prognostic information in addition to the clinical variables identified in earlier regression analyses.

The final model for PIQ, taking clinical and imaging variables into account, suggests that the best predictors are TOMI error scores; the number of VBM-detected grey matter abnormalities (with widespread VBM abnormalities being predictive of a decrease in PIQ) and hippocampal abnormalities identified on visual inspection of MR images. Once imaging variables were added to the model, the perinatal variables contained in PCA_1 (gestational age at birth, birth weight, duration of oxygen supplementation) no longer contributed significantly to prediction of PIQ. Interestingly, periventricular white matter abnormalities were only predictive of PIQ as long as TOMI score was not considered. This is likely to be explained by the TOMI scores and periventricular white matter abnormalities being strongly related as indicated by univariate analyses. The regression analyses indicate, however, that the independent effect of white matter lesions in the periventricular region on performance on tests on the PIQ scale was not as large as the independent effect of the degree of impairment of motor function (indicated by the TOMI error scores), which is not surprising since most of the tests on the PIQ scale are timed and also involve use and fine motor co-ordination of the hands. However, since TOMI scores can only be obtained when the child is old enough to perform the Test of Motor Impairment (i.e.>= 4 years of age), and VBM analysis of MR data is time consuming and often complicated, the identification of periventricular white matter abnormalities on visual inspection of MR images, in particular, in combination with visual assessment of the hippocampi on MRI, can serve as an early predictor for PIQ that is relatively easy to obtain in a routine clinical setting.

The final model for VIQ, taking clinical and imaging variables into account, suggests that the best predictors are in fact a composite of perinatal variables (gestational age,

birth weight, duration of oxygen supplementation) combined with findings from visual inspection of MR images (grey matter abnormalities, in particular hippocampal abnormalities). These variables, too, are relatively easy and early to obtain in a routine clinical setting, thus the findings from these analyses might improve early prediction of cognitive outcome. However, the findings from this study can not necessarily be generalised. The results have to be interpreted in the context of the particular group that was examined in this study. The study group is not a random sample and some variables that have been shown in previous research to influence cognitive outcome in preterm children, e.g. early nutrition (Lucas, Morley and Cole, 1998), environmental and sociodemographic factors (Hack et al, 1992; Taylor et al, 2004), were not examined. It would be of great interest to investigate further whether and, if so, how such variables are associated with cognitive outcome in a study population in which a large proportion has brain lesions sustained in the context of preterm birth.

10.6.4 Methodological considerations and limitations of the analyses performed

There are a number of methodological issues and some limitations regarding the analyses described in this chapter, and these have to be considered in the interpretation of the results. First, there are some points to be considered regarding the neuroimaging data; this has been discussed in detail in chapter 9, section 9.5.7.

Second, in some of the univariate analyses the number in subgroups that were investigated were small and therefore the results from these statistical tests need to be interpreted with this in mind. The exclusion of 14 children from the analyses (for details see introduction to this chapter) might introduce a bias into both the univariate and the regression analyses since these excluded children either had such severe motor impairment that they were not able to complete the required number of subtests on the WISC-R needed for computation of an IQ score (8/10 that were excluded because of missing WISC-R data) and/or had large lesions and/or abnormally shaped brains so that they had to be excluded from the VBM analyses (5/9 datasets that were not included in the analyses dealing with VBM data). The majority of the excluded children had epilepsy. Therefore, it is possible that a bias has been introduced towards

underestimation of both the effect of brain lesions and the effect of epilepsy on cognitive function.

It has been shown in previous studies that in preterm children specific cognitive deficits are more frequent than in term born children. Examination of cognitive function in the current study was limited to Performance IQ and Verbal IQ as indicated the WISC-R, which does not allow detailed investigation of specific cognitive deficits. Since the main focus of this study is on investigation of epilepsy and overall cognitive function and the neural correlates as indicated by neuroimaging findings, the investigation of specific cognitive deficits is beyond the scope of this thesis.

In the current study, assessment of brain structure and identification of abnormalities is based on visual inspection of MR images for white and grey matter, and on VBM analysis for detection of subtle grey matter abnormalities that might go undetected on visual inspection. No voxel based assessment of white matter structures based on 3D MR data sets and/or based on diffusion imaging data was included in the protocol since the main hypothesis of this thesis was that in preterm children with epilepsy and/or cognitive impairment subtle grey matter abnormalities are present in addition to visible white matter lesions. Recent studies in typical populations (e.g. Schmithorst et al, 2005; Deary et al, 2006) and also in preterm populations (e.g. Skranes et al, 2007; Constable et al, 2008) have shown that white matter integrity as indicated by parameters derived from diffusion MRI is associated with cognitive function. It is likely that further investigation of brain structure with diffusion MRI in the group of children that are the subject of the current study would add interesting information regarding associations between cognitive outcome and more subtle white matter abnormalities.

10.7 Conclusions

Some methodological issues that may influence the analyses have to be considered and thus, similarly to the results of the analyses investigating associations between imaging variables and epilepsy, the findings should be regarded as tentative. Nevertheless, the analyses presented in this chapter showed strong associations of brain injury, as indicated by visual inspection of MR images and VBM analysis of grey matter, with cognitive outcome. A positive correlation between the degree of periventricular white matter reduction and decrease in both VIQ and PIQ was found, with PIQ being more affected than VIQ. Similarly, the presence of grey matter abnormalities on visual inspection of MR images, and, within this category hippocampal abnormalities, was associated with decrease in both IQ scores. In those children in whose data sets subtle grey matter abnormalities were detected by VBM analysis, IQ scores were lower than in those without VBM-detected grey matter abnormalities. This was, however, only significant for the correlation between PIQ and the number of VBM-detected abnormalities, not for VIQ. The analyses presented suggest that, in this group of preterm children, in addition to periventricular white matter reduction and/or grey matter abnormalities that can be identified on visual inspection, widespread subtle grey matter abnormalities contribute to the explanation of cognitive outcome.

The findings suggest that the effect of the presence of epilepsy on cognitive outcome is not significant once brain lesions identified on visual inspection of MR images, in particular the extent of lesions, have been accounted for. However, a weak, non-significant effect of epilepsy on PIQ was seen in an exploratory analysis examining the effect of epilepsy and VBM-detected grey matter abnormalities. Overall, however, the findings indicate that in this study group epilepsy can be regarded as a marker of the severity of brain injury rather than having a significant independent effect on cognitive function.

The results of the regression analyses suggest that in this sample imaging variables from both visual inspection of MR images and VBM analysis of grey matter indeed contributed to and improved significantly the prediction of cognitive outcome compared to using clinical variables alone.

Part V

General discussion, conclusions and future directions

In this last part of the thesis a summary of the main findings is given. Some methodological considerations are outlined and directions for future work are indicated. The main conclusions from the work presented in this thesis conclude this part.

Chapter 11: Summary of main findings, methodological considerations, future directions and conclusions

The work described in this thesis aimed to investigate characteristics and neural correlates of epilepsy and cognitive function in a group of children born before 33 weeks of gestation. The main hypothesis was that epilepsy and/or cognitive impairment are associated with brain grey matter abnormalities that are additional to the white matter abnormalities typically seen in preterm children. This was investigated by combining visual analysis of MR images with a statistical image analysis method (voxel-based morphometry) for detection of subtle abnormalities of grey matter. In addition, it was investigated whether perinatal variables, neonatal variables and/or data obtained from neurological examination were associated with epilepsy and cognitive outcome. Finally, associations between brain lesions, epilepsy and cognitive outcome were explored.

11.1 Summary of main findings

Visual analysis of MR images identified brain abnormalities that had gone undetected on neonatal ultrasound. In addition, lesions that had been detected on neonatal ultrasound, could be characterised in more detail by MR imaging. Voxel-based morphometry identified subtle abnormalities (which were either focal or widespread) in grey matter that had not been detected on purely visual analysis of MR images. Widespread VBM-detected grey matter abnormalities were associated with periventricular white matter reduction identified on visual inspection of MR images.

Univariate analyses provided evidence for an association of epilepsy with the degree of periventricular white matter reduction and with VBM-detected grey matter abnormalities (in particular, when widespread), suggesting that, indeed, in those with epilepsy additional grey matter lesions were present that might predispose to epilepsy. Regression analyses suggested that, although VBM analyses of grey matter improved

prediction of epilepsy, the best independent predictor for epilepsy was the degree of periventricular white matter reduction on visual inspection of MRI.

Univariate analyses investigating associations between IQ scores and neuroimaging findings provided evidence for an association between the degree of periventricular white matter reduction and both PIQ and VIQ. Furthermore, grey matter abnormalities, in particular hippocampal abnormalities detected on visual inspection of MR images, were associated with a decrease in both IQ scales. A significant association with VBM-detected grey matter abnormalities was found for PIQ only. Regression analyses identified both neuroimaging variables and clinical variables as predictors for IQ scores. For VIQ, a composite score containing the variables gestational age at birth, birth weight, and oxygen supplementation beyond 37 weeks gestational age, as well as grey matter abnormalities on visual inspection of MR images were identified as predictors. For PIQ, the TOMI score (measure of motor impairment), hippocampal abnormalities on visual inspection of MR images, and VBM-detected grey matter abnormalities were identified as predictors.

In summary, the investigations performed in this thesis identified previously undetected grey matter abnormalities in a considerable proportion of the study population. The subtle grey matter abnormalities detected by VBM analysis can be best interpreted within the context of the neuropathological studies (described in chapter 3), which suggest that a) subsequently to a primary injury to periventricular white matter secondary widespread developmental changes in primarily undamaged grey matter occur (Marin-Padilla, 1997, 1999, 2000), and b) injury to the periventricular white matter can be associated with damage to grey matter structures such as the cerebellum, basal ganglia/thalamus, hippocampi, and brain stem (see e.g. Friede, 1989; Paneth et al, 1994). The analyses provide converging evidence for epilepsy and cognitive impairment to be related to both periventricular white matter lesions and subtle grey matter abnormalities. Those children with normal MRI on visual inspection and without VBM-detected grey matter abnormalities had good cognitive function. The worst outcome was seen in those with periventricular white matter reduction associated with VBM-detected grey matter abnormalities. Using combined information from visual inspection of MR images and VBM analysis of grey matter indeed significantly improved the prediction

of epilepsy and overall cognitive outcome in comparison with using information from clinical variables alone.

11.2 Epilepsy and cognitive function

One of the questions that this study aimed to investigate was the effect of the presence of epilepsy on cognitive function. Although the children in this study had mild epilepsy, IQ scores were lower in the group with epilepsy when compared to the group without epilepsy, and this was most pronounced for PIQ. However, once brain injury, as indicated by findings from neuroimaging, was taken into account, the significant effect of epilepsy on IQ scores was no longer seen. The analyses that were performed in this study for investigation of the effect of epilepsy on cognitive function therefore suggest that in this group of preterm children, the brain injury rather than epilepsy has a main effect on cognitive outcome. This interpretation is supported by the finding that those with epilepsy and normal MRI findings had on average IQ scores in the normal range and no clear abnormal neurological findings. Thus, epilepsy might be regarded as a marker for the severity of the brain injury, and more specifically, for the extent of grey matter damage.

11.3 Methodological considerations

There are a number of methodological issues that need to be kept in mind in the interpretation of the findings from this study.

Preterm control subjects without epilepsy had been chosen to achieve balance with respect to white matter ultrasound abnormalities. This design was chosen since the primary hypothesis was that in those children with epilepsy and/or cognitive impairment, grey matter lesions, additional to the white matter abnormalities, were present. Such a design could, however, result in a reduction in the observed associations between some independent variables of interest and the outcome epilepsy. Explorative statistical analyses, which were performed to test for such an effect, indicated that the study design did not significantly affect the results obtained from analyses that

examined associtations between imaging findngs and epilepsy. Nevertheless, it needs to be kept in mind that the study group is not a random sample. Thus, generalisation of the results from the statistical analyses is not possible.

Information on seizure semiology was obtained from the parents by interview and information from EEG was based on interictal recordings that did not include a sleep recording. Based on this information, it was difficult in a number of cases to distinguish between focal onset of seizures and primary generalised onset of seizures. In the investigation of a population where focal brain pathology is assumed and thus focal onset seizures might be expected, it would be important to improve the methods for determination of a focal onset of seizures for more detailed examination of associations with imaging findings.

Analysis of MR images included two methods, visual inspection and voxel-based morphometry. Visual inspection of images is subjective and although there was good agreement between the observers in judging the degree of white matter reduction, this is not an accurate measure. However, in a routine clinical setting, when, for example, time may be limited and quantitative analysis methods are not easily available, using information from visual inspection of images provides valuable information with regard to prediction of outcome as demonstrated by this study

In this study, VBM was used in a paediatric population in which a number of subjects had visible brain lesions. Images were normalised to the standard adult template in SPM and single subject versus group comparison was performed. There are a number of methodological issues that can influence the findings from VBM analysis, such as the use of an adult template for analysis of paediatric data, possible misclassification of tissue in those datasets with large lesions, limitation of sensitivity for detecting abnormalities, and the single subject versus group design. A number of attempts have been made to adapt and optimise the VBM procedure in order to minimise the effect of these factors. These methodological issues have been discussed in detail in chapter 8, section 8.4.4. In conclusion, the findings from the VBM analyses, in particular in those datasets with large visible lesions, have to be interpreted with caution. However, the results of the VBM analysis performed in this study are consistent with findings from previous studies, supporting the view that the detected grey matter abnormalities in the

majority of the cases most likely reflect true biological differences between the preterm brain and the brains of a group of healthy controls.

11.4 Future directions

The findings of the investigations described in this thesis have created a number of questions that could provide a basis for future studies.

First, for a more detailed examination of aspects of epilepsy in preterm children, larger scale prospective studies should be conducted. It would be of interest, for example, to investigate in more detail the long term impact of neonatal seizures on outcome in preterm children. In the current study, this was possible only to a very limited extent. Prospective larger scale, possibly longitudinal, studies would make it possible to investigate in more detail the natural course of epilepsy in such a population in which a fairly homogeneous brain pathology can be presumed (i.e. hypoxic-ischaemic/inflammatory or haemorrhagic brain lesions acquired in the context of preterm birth). In addition, longitudinal studies would allow investigation of possible effects of epilepsy and characteristics such as seizure frequency, seizure type, and medication on cognitive outcome in more detail when combined with serial assessments of development and cognitive function. More comprehensive EEG studies might allow better determination of focal onset seizures, laterality, and localisation of a seizure focus and more detailed investigation of associations of such variables with imaging findings.

Second, studies that expand the psychometric test protocol to include not only Performance and Verbal IQ, but also tests that focus on examination of more specific cognitive abilities might make it possible to investigate the effects of epilepsy on cognitive function in much more detail. In the current study, it has been shown that performance on IQ testing was influenced by motor impairment. Therefore, in preterm infants, who often have motor problems, the use of tests that are less dependent on motor function might provide a better reflection of these children's cognitive abilities.

Third, the imaging methods could be refined and the protocol could be expanded, which would allow investigation of brain structure in even more detail than in the current

study. For example, the use of more recent versions of SPM for conducting voxel-based morphometry should provide better results in the pre-processing of data sets of brains with abnormal shape or large lesions. White matter abnormalities were assessed visually in the current study. MR diffusion imaging and quantitative analysis of MR diffusion data was not part of the protocol. Including MR diffusion imaging for investigation of white matter structure and associations with outcome, in particular cognitive outcome, would be of great interest in a preterm population and would also improve the understanding of the long term consequences of the more subtle forms of injury to white matter.

11.5 Conclusions

The work described in this thesis has documented patterns of epilepsy and overall cognitive function in a group of very preterm children. The study has demonstrated that in these children both white matter and grey matter abnormalities are present and that these abnormalities are related to outcome. The study has shown that adding a quantitative MR image analysis method improves detection of brain abnormalities and delineation of the extent of brain injury sustained in the context of preterm birth. Importantly, it has been demonstrated that using information from imaging alongside clinical variables, and, in particular combining information from qualitative and quantitative image analysis improves the prediction of outcome. It has also been shown that in the population under investigation, the extent of brain injury has a stronger effect on cognitive outcome than the presence of epilepsy.

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Appendix 1: Details of excluded subjects and missing data (tables A1-A3)

Table A1: Gender, perinatal and neonatal characteristics of the children who were not included in the final study (no consent for MRI, EEG after clinical assessment and interview)

ID	Birth weight (g)	GA (weeks)	Gender	Epilepsy	Multiple birth	APGAR 5 min	Duration of oxygen suppl. (weeks GA)	PDA
OL	1435	30	male	yes	no	9	>37	yes
JMG	940	29	male	yes	no	3	>37	yes
AlO*	1600	29	male	yes	no	9	<37	no
CHT	770 (SGA)	25	male	yes	no	8	<37	yes
ADI	1099	26	male	no	no	8	>37	yes
DYK	1454	28	male	no	no	8	<37	no
AND	1270	29	male	no	no	4	>37	yes

SGA= small for gestational age, GA= gestational age, AED = antiepileptic drug, PDA = persistent arterial duct

*severe neonatal meningitis

Table A2: Neonatal cranial ultrasound findings, neurological status, seizure type, age at onset of seizures and medication for treatment of epilepsy of the children who were not included in the final study (no consent for MRI, EEG after clinical assessment and interview)

ID	Neonatal cranial ultrasound findings	Neurological status/sensory impairments	Seizure type	Age of onset of seizures (months)	Seizure frequency
OL	IVH II bil, mod/severeVD	leg dominated bilateral spastic CP/ severe hearing loss	seizure free, previous seizures: primary generalised	60	seizure free without AED
JMG	IVH III r, HPI l	three limb dominated bilateral spastic CP	complex partial	36	1-3 seizures /month; 1 AED
AlO*	GLH l, bright echoes bil	leg dominated bilateral spastic CP/severe hearing loss	partial seizures, secondary generalisation	48	1-3 seizures /year; no AED
CHT	HPI r, GLH l, mod/severeVD	leg dominated bilateral spastic CP/ visual impairment	seizure free, previous seizures: primary generalised	60	seizure free without AED
ADI	IVH III l, HPI r, bright echoes bil ++, mild term VD	normal	no epilepsy	-	-
DYK	GLH bil, mild trans VD	normal	no epilepsy	-	-
AND	HPI r, IVH III l, bright echoes ++ r, bil mod term VD	suspicious (increased muscular tone legs)	no epilepsy	-	-

IVH = intraventricular haemorrhage (grading, see appendix 3), GLH = germinal matrix haemorrhage, HPI = haemorrhagic parenchymal infarction, VD = ventricular dilatation, CP = cerebral palsy, AED= antiepileptic drug

Table A3: Summary of missing data of the children included in the final study (does not include MRI data that had to be excluded because of artifacts. This is shown in appendix 7)

Assessment	**Subject (group)**	**Reason**
EEG	NSi (no epilepsy) LiWi (no epilepsy) JSk (no epilepsy) DSk (no epilepsy) SDaW (no epilepsy) JC (no epilepsy)	data lost data lost data lost data lost data lost data lost
TOMI (Test of motor impairment)	TM (epilepsy)	no routine follow-up at age 8 years at UCH and refused at time of current study
WISC-R (Wechsler Intelligence Scale for Children-Revised)	JC (no epilepsy) TM (epilepsy)	no routine follow-up at age 8 years at UCH no routine follow-up at age 8 years at UCH

Appendix 2: Data collection sheets and scoring forms

Data collection sheet for study "Epilepsy in preterm children "

ID **Surveyno UCL** **GOS Hospitalnumber**

Family history	**yes**	**no**	**comment**
Consanguinity			_____
Siblings			_____
Neurological disease			_____
Motor disorder			_____
Mental retardation			_____
Chromos.abnormal.			_____
Epilepsy			
relat. grade _____			
type of epilepsy_____			
Febrile convulsions			
relat. grade_____			

Maternal history
Age (pregnancy) _____

Previous pregnancies		
Miscarriages/stillbirths		
Previous preterm births		
Chronic disease		
since _____		
medication _____		
Epilepsy/history of seizures		

Pregnancy			
Smoking			_____
Drugs/alcohol			_____
Medication			_____
Bleeding			trimester_____
Preterm labour			_____
Signs for pre-eclampsia			_____
Premature rupture of membranes			_____
Signs for infection (chorioamnionitis.)			_____
Signs of prenatal infection (TORCH)			_____

Peri and neonatal data
Cause of prematurity _____

Gestational age _____weeks

Delivery spontaneous
forceps
vacuum

		el.caes.section ☐
		em.caes.section ☐
	Presentation	cephalic ☐
		breech ☐
Multiple birth		first ☐
		second/third ☐
Birth weight	_____g	centile_____
length	_____cm	
head circumference	_____cm	
Apgar		1`☐ 5`☐ 10`☐
Umbilical cord pH	_____	
Blood gases within 2 hrs	_____	
Need for resuscitation	☐	
Need for ventilation	☐	number of days _____
		complications _____
Sepsis	☐	
Neonatal convulsions	☐	(details see below)
Apnea	☐	
Hypoglycemia	☐	
Jaundice	☐	day of onset _____
		phototherapy ☐
Feeding problems in neonatal period	☐	
Medication in neonatal period	_____	
Other problems in neonatal period (including surgery)	_____	

Developmental milestones/behaviour (age corrected for gestational age)

Motor development

sitting	_____	months
crawling/shuffling	_____	
walking	_____	
no independent walking	☐	

Language development

words	_____	months
sentences	_____	months

Communication _____

_____

Behavioural problems _____

_____

If yes,	minor	☐
	mild	☐
	severe	☐

Sleeping problems _____

_____

If yes, minor
mild
severe

Feeding problems

If yes, with help
tube feeding

School performance

school report
normal
teacher dissatisfied
referred Ed Psych
extra help
repeating a year
special school

Medical history beyond the neonatal period

Severe infections
History of meningitis
History of encephalitis
History of brain injury
Shunt revisions_____

History of seizures

Neonatal seizures day of onset_____

Connected with hypoglycemia
sepsis
encephalitis
meningitis
haemorrhage
metabol.disorder

Treatment _____

Convulsions with fever
age first conv _____
antecedent _____
temperature _____
cause of fever known

First seizure simple |
complex |

Further convulsions with fever | age_____months
simple |
complex |

History of status (PFC) |

Seizures associated with metabolic/toxic events |

_____

Non-febrile seizures

Age of first seizure _____months

History of status |

Frequency 1-3/year 4-11/year 1-3/month

1-6/week 1-3day 4-10/day

>10/day

Longest period seizure free _____months
Seizure free since _____months

Currently seizure free

Description of seizures

(setting, provocation, warning/early phase, motor aspects (type of movement distribution), sensory aspects (type, distribution), responsiveness, colour change, recovery phase, length)

First seizure

Subsequent seizures

Current seizures

Worst seizure

Treatment	**previous**	**present**
No treatment		
Carbamazepine		
Phenobarbitone		
Sodium Valproate		
Phenytoin		
Vigabatrin		
Ethosuximide		
Lamotrigine		
Clobazam		
Clonazepam		
Nitrazepam		
Gabapentin		
Steroids		
Ketogenic diet		
Other		
Intractable		

Neurodevelopmental assessment age 8 years

Neuromotor status

Normal
Impairment, no disability.
Impairment with disability.

Cognitive function age 8 years (WISC-R) FSIQ PIQ VIQ

Vision (tested at age____) _____
Hearing (tested at age____) _____

Examination (epilepsy study)

Weight centiles
Height
Head circumference

Dysmorphic features
Deformities

General examination
normal
any abnormal findings
_____
_____

Neurological examination
normal
suspicious
abnormal

Cranial nerves _____

Bulbar involvement

Mobility _____

Gait _____

Posture _____

Speech _____

Communication _____

Hand function
(describe)
left_____
right_____

	left	right
Muscle tone (passive)		
upper limbs		
lower limbs		
Muscle tone (active)		
upper limbs		
lower limbs		
Rigidity		
upper limbs		
lower limbs		
Spasticity		
upper limbs		
lower limbs		
Reflexes		
upper limbs		
lower limbs		

Ataxia	upper, lower limbs, trunk, face,left, right.....
Dyskinesia	
athetosis	
dystonia	
tremor	
chorea	
myoclonia	

Classification of "CP-syndromes"		**Subgroups of CP syndromes**
Spastic CP	unilateral (hemiplegia)	
	bilateral	leg dominated three limb dominated four limb dominated
Ataxic signs		
Dyskinetic signs		

Imaging

Neonatal cranial ultrasound scan

			left	right
Normal				
Abnormal				
PVH		GLH/choroid pl.		
		GMH/subep. cysts >7 d		
		II		
		III		
		IV (=HPI)		
HPI				
SEPC				

		left	right
PVL			
	bright echos +		
	bright echos ++		
	cystic PVL		
Flares			
Ventricular dilat. (present at term)	mild		
	moderate		
	hydrocephalus		
Shunt			
Atrophy	general.		

Scoring form for visual inspection of MR images

MRI findings **Study "Epilepsy in preterm children"**

Surveyno UCL DOB Patient/Control

GOS Hospitalno

Scan details Date axT2 corT2 MPRAGE

Hippocampi	left	normal	small	abnormal signal
	right	normal	small	abnormal signal
Corpus callosum	rostrum	normal	small	
	genu	normal	small	
	body	normal	small	

Basal ganglia_____

Cerebellum_____

Cortex_____

White matter *PVL* = *gliosis* parietal-occipital_____
centrum semiovale_____
adj. to frontal horns_____

+ *wmred*
focal_____
global_____
severe(-subcortical)_____

WMR only, no gliosis
unilateral/focal_____
bilateral_____
severe (- subcortical)_____

Miscellaneous (describe)

Brainstem/visual pathway/olfactory/ pituitary _____
Myelin pattern _____

Scoring form for seizure type and epilepsy diagnosis

Seizure type and epilepsy diagnosis **Study "Epilepsy in preterm children"**

ID Surveyno

Neonatal seizures

Convulsions with fever age
prolonged febrile seizures

Seizures associated with toxic/metabolic events age

Family history of seizures/epilepsy

Non-febrile seizures age first seizure

seizure free since

frequency

Treatment previous
current

Description of first seizure

Current seizures

Worst seizure

Classification of seizures

Partial seizures

motor signs	tonic	
	clonic	
	automatisms	
sensory signs	visual	
	olfact/gustator.	
	auditory	
somatosens.		
psychic signs	dysphasia	
affective		
	déjà vu	

loss of consciousness

postictal signs

secondary generalisation

Generalised seizures	tonic-clonic		focal signs no focal signs
	tonic		
	myoclonic		
	atonic		
	infantile spasms		focal signs no focal signs
	absences	simple additional signs (clonic, tonic, atonic,automatisms)	

Seizure classification:

1.
2.
3.

Scoring form for EEG findings

EEG Report Study "Epilepsy in preterm children"

Surveyno UCL	DOB	Date assessment

Patient
Control

Current treatment

Events observed during recording	yes no	awake drowsy asleep

General activity_____

Abnormal activity	general focal multifocal	frontal central temporal parietal occipital

Isolated sharp-waves/spikes

Sharp-slow waves/spike waves complexes

Overbreathing

Photic-stimulation

Conclusion	normal abnormal

Appendix 3: Definitions of neonatal cranial ultrasound abnormalities

-Uncomplicated germinal matrix/intraventricular haemorrhage (GLH/IVH): Haemorrhage into the germinal layer (matrix) or lateral ventricle, including SEPC but not associated with PVF, nor with ventricular dilatation with CSF, nor HPI, nor loss of brain tissue.

-Subependymal pseudocyst (SEPC): Cystic degeneration within a germinal layer hemorrhage without cystic changes in the surrounding brain parenchyma.

-Haemorrhagic parenchymal infarction (HPI): Markedly increased echodensities within the brain parenchyma, wedge shaped and extending from the ventricular margin, in association with an ipsilateral germinal matrix/intraventricular haemorrhage.

-Periventricular flare (PVF): Transient abnormally increased echodensities in the periventricular white matter without an ipsilateral germinal matrix/intraventricular haemorrhage.

-Cystic periventricular leukomalacia (cPVL): Cystic lesion within the periventricular white matter, not preceded by HPI at the same site.

-Ventricular dilatation (VD): Dilatation of the lateral ventricle with CSF such that the depth of the frontal horn immediately anterior to the thalamo-caudate notch is greater than 3mm (97^{th} centile).

-Post-haemorrhagic hydrocephalus: Marked pressure-driven dilatation of a lateral ventricle with CSF such that its width is 5mm or more above the 97^{th} centile for this.

Appendix 4: Neonatal cranial ultrasound findings of the study participants

Table A5: Neonatal cranial ultrasound findings for each study participant

ID	Gender	BW (g)	GA (weeks)	Epilepsy	Individual neonatal ultrasound findings	Ultrasound category
TR	M	1078	29	+	normal	normal
TM	F	1300	31	+	normal	normal
TO	M	1600	31	+	normal	normal
AP	M	1316	28	+	bright echo + bil; mod,trans VD	VD (non-parenchymal)
SSk	M	879	26	+	GLH bil; mod,trans VD	non-parenchymal
LB	M	1500	30	+	mild,trans VD	VD (non-parenchymal)
BK	M	637	24	+	HPI right,	parenchymal
TC	F	1214	28	+	IVH II bil	non-parenchymal
PD	M	1060	30	+	HPI r, mild VD	parenchymal
WS	M	1759	32	+	GLH l	non-parenchymal
SD	F	890	26	+	IVH II bil; mild,trans VD	non-parenchymal
HJ	M	963	26	+	HPI l	parenchymal
LR	M	560	24	+	HPI r, IVH III l; bright echo + bil; mod,term VD	parenchymal
PP	F	847	26	+	HPI l, IVH III r	parenchymal
JW	M	1220	26	+	cPVL bil	parenchymal
AM	F	634	23	+	cPVL r;	parenchymal
SH	M	1830	31	+	GLH l	pon-parenchymal
KS	F	793	26	+	HPI r, IVH III l	parenchymal
ML	M	1392	27	+	IVH III bil; cPVL l	parenchymal
AU	M	1798	30	+	IVH II l; bright echo + bil	non-parenchymal
SHay	M	1104	27	+	HPI r, IVH II l, mod,term VD	parenchymal
EG	F	836	26	+	HPI l, IVH II r	parenchymal
TS	M	1036	27	+	HPI l, GLH r; bright echo ++ r	parenchymal
EF	F	647	28	+	cPVL bil	parenchymal

RK	M	1706	31	-	GLH l	non-parenchymal
DS	M	1480	32	-	normal	normal
JoCol		1436	31	-	cPVL bil	parenchymal
AT	M	690	24	-	IVH II bil	non-parenchymal
AC	F	1620	30	-	IVH II r, GLH l,bil; bright echo +	non-parenchymal
CS	M	1200	29	-	cPVL bil	parenchymal
VH	F	1124	27	-	IVH III bil; mild trans VD	non-parenchymal
CR (I)	F	1215	29	-	IVH II l	non-parenchymal
MD	M	1159	27	-	mod,trans VD	VD (non-parenchymal)
JSk	M	1132	27	-	normal	normal
ASa	F	864	26	-	mild,trans VD	VD (non-parenchymal)
SDaW	F	695	25	-	normal	normal
BA	M	1229	26	-	GLH bil,mild,trans VD	non-parenchymal
SF	F	1478	31	-	normal	normal
JRu	M	714	25	-	HPI l; bright echo + l	parenchymal
BBe	F	1432	28	-	IVH II l, GLH r; bright echo + r	non-parenchymal
DSk	M	1192	27	-	IVH III l, IVH II r, bright echo + bil	non-parenchymal
LiWi	F	1055	31	-	GLH bil; bright echo + bil	non-parenchymal
NK	F	1660	31	-	IVH II r, GLH l; bright echo + bil; mod trans VD	non-parenchymal
LWa	F	1804	32	-	GLH bil	non-parenchymal
NSi	F	1730	32	-	GLH bil; bright echo ++ bil cPVL bil	parenchymal
GO	M	890	24	-	HPI r, IVH II l	parenchymal
DHay	M	1000	27	-	severe VD	parenchymal
CT	M	1040	28	-	IVH III bil;cPVL r	parenchymal
MC	M	769	24	-	HPI l; IVH II r; mod,trans VD	parenchymal
LH	M	1470	29	-	cPVl l, bright echo ++ r	parenchymal
RR(II)	F	1630	29	-	HPI l	parenchymal
LO(I)	F	933	27	-	HPI l, IVH III r	parenchymal
CO(II)	F	997	27	-	HPI l, IVH II r; bright echo + bil	parenchymal
JC	M	918	26	-	HPI l, IVH III r	parenchymal

GLH = germinal matrix haemorrhage, IVH = intraventricular haemorrhage, HPI = haemorrhagic parenchymal infarction; PVL = periventricular leukomalacia. cPVL = cystic periventricular leukomalacia, VD = ventricular dilatation, r = right, l = left, bil = bilateral, trans = transient, mod = moderate

Appendix 5: Details of each subject's neurological status, IQ scores, seizure types, EEG findings, findings from visual assessment of MR images and summary of findings on voxel-based morphometry analysis

Table A5: Neurological findings, seizure type, EEG findings, findings on visual inspection of MR images, summary of VBM grey matter analysis for each subject

ID	**Neurological status and additional impairments; FSIQ, PIQ,VIQ[$]**	**Seizure type**	**EEG** - *epileptic discharges** - diffuse/focal abnormal background activity and/or isolated sharp waves/spikes*	**Visual assessment of MRI***	**VBM Number of peaks (0, 1-2, >=3)**	**VBM Location of peaks****	**More (↑)/less (↓) grey matter in subject than in VBM controls*****
TR	Suspicious 96 78 111	Typical absences	*Overbreathing/Photic stimulation: Frontal bilateral, starting on right*	Normal	0	-	-
TO	Normal 85 97 77	Complex-partial	*Generalised, starting on right side*	Normal	0	-	-
SD	Suspicious 86 95 80	Atonic	Isolated sharp waves mid-parietal (left>right) Central regions, occipital	MWMR left (po) HC bilateral small	0	-	-
TM	Normal -	Complex partial Second. generalised	*No abnormality*	Normal	1-2	Insular gyrus left	↓
SSk	Suspicious 106 92 117	Atonic Simple partial Atyp absences	*No abnormality*	Normal	1-2	Cingulate gyrus left	↑
LB	Suspicious 64 61 72	Generalised clonic Atyp. absences	Isolated slower transients temporal/temporo-parietal bilateral	MWMR bilateral (po) HC bilateral small	1-2	Hippocampus left	↓
BK	Suspicious	Generalised myoclonic	Slow activity fronto-temporal left>right	BG (caudate right)	1-2	Superior temporal gyrus right	↓

	63 70 60	(neonatal seizures)					
TC	Normal 92 96 90	Generalised tonic-clonic Atpy. absences	Intermittent sharp theta activity frontal bilateral	MWMR bilateral (cs)	1-2	Superior parietal gyrus right	↓
SH	BSCP three limb dominated (left>right) 96 86 106	Complex-partial Second. generalised	*Parietal right* Isolated sharp transients mutlifocal bilateral	SWMR bilateral (left>right, po) Gliosis (po)	1-2	Cingulate gyrus right Cingulate gyrus left	↑
PD	BSCP four limb (left>right) Mild dykinesia 47 45 67	Generalised clonic	*Photic stimulation:Frontal bilateral (left>right)* Isolated sharp waves frontal bilateral (right >left) Slow activity frontal bilateral	MWMR bilateral (left>right,cs) Gliosis (cs) Cerebellum small bilateral	>=3	Thalamus right Thalamus left Cerebellum right Cerebellum left Temporal lobe (region of middle temporal gyrus) right	↑ ↓
WS	BSCP three limb dominated (right>left) Hearing impairment 74 45 105	Generalized tonic Atyp.absences (neonatal seizures)	Isolated sharp waves anterior/mid-temporal left	MWMR bilateral (left>right,po) Gliosis (po,cs)	>=3	Occipital lobe (near collateral sulcus) right Thalamus right Cerebellum left Frontal lobe (region of inferior frontal gyrus) right Postcentral gyrus right Parietal lobe (superior parietal) right	↑ ↓
LR	Suspicious 59 52 70	Complex-partial Atyp. absences	Isolated sharp waves fronto-central (Sylvian region) right, left central	SWMR bilateral (right>left, po)	>=3	Thalamus right Thalamus left Region of putamen right Parietal lobe right (region of parieto-occipital fissure) Hippocampus right	↑ ↓

						Temporal lobe (region of middle temporal gyrus) right	
JW	BSCP leg dominated (left>right) Horizontal nystagmus -	Infantile spasms Generalised myoclonic (neonatal seizures)	*Frontal bilateral (left>right)* Isolated sharp waves mixed with slow activity frontal bilateral	SWMR bilateral (po) schizencephaly bilateral , adjacent polymicrogyria HC small bilateral	>=3	Thalamus left Fronto-parietal right; (region of schizencephaly) Thalamus right Fronto-temporal left; (region of schizencephaly) Frontal lobe left (adjacent to schizencephaly) Parietal lobe left	↑
						Temporal lobe right Temporal lobe left Frontal left (region of schizencephaly) Parietal lobe left Parietal lobe right Fronto-partietal (region of schizencephaly) Cerebellum left Insula left	↓
AM	Spastic hemiplegia left Arm more affected than leg 66 46 64	Infantile spasms Atyp absences (neonatal seizures)	Isolated sharp waves right central and frontal Intermittent slow activity temporal bilateral	SWMR right (po,cs) Gliosis (po, cs) Cortex parietal right (shunt)	>=3	Parietal lobe (region of precentral gyrus) right Region of cingulate gyrus/sulcus right	↑
						Temporo-parietal right (region of shunt)	↓
ML	BSCP four limb (symmetrical)	Infantile spasms Atyp absences (neonatal	*Left central-parietal*	SWMR bilateral (left > right, po,cs,f)) gliosis (po,cs)	>=3	Thalamus left Thalamus right Temporal lobe left adj. to MCA region	↑

	Ataxia, mild dystonia Severe visual impairment -	seizures)		Cortex (MCA left) Cerebellum and pons small HC left small		Parietal lobe left Cerebellum left Cerebellum right Temporal lobe left, MCA region Parietal lobe left (not adj. to MCA region) Amygdala/hippocampus left	↓
AU	BSCP leg dominated (left>right) Mild ataxia trunk -	Complex-partial Atyp. absences	Isolated sharp waves left and right inferior frontal (left>right) Intermittently slow activity temporal and frontal bilateral (left>right)	SWMR bilateral (po,cs,f) HC abnormal signal right	>=3	Thalamus right Thalamus left Temporal lobe (region of superior temporal gyrus) right Frontal lobe (region of middle frontal gyrus left Cingulate gyrus left	↑ ↓
EF	Spastic hemiplegia right -	Complex-partial Absences	*Bilateral generalised (left>right)* Intermittent slow activity generalised both hemispheres	SWMR bilateral (left >right, po,cs,f) Gliosis (po,cs)	>=3	Frontal lobe (region of inferior frontal gyrus) left Temporal lobe (region of superior temporal gyrus) right Parietal lobe bilateral Cerebellum left Interhemispheric fissure	↑ ↓
AP	Normal 124 119 122	Complex-partial Secondary generalisation	*Bilateral generalised*	Normal	-	No VBM analysis performed	-
KS	BSCP leg dominated (left>right)	Complex-partial Atyp. absences	Isolated sharp waves frontal, fronto-temporal right>left, central Intermittent slow activity	SWMR bilateral, (right > left; po, f), gliosis (po,f)	-	No VBM analysis performed	-

	47 46 57		frontal/fronto-temporal right>left	Putamen,Thalamus bilateral Cortical lesion (Ulegyria) right temporal HC small right Cerebellum small left			
SHay	BSCP leg dominated (left>right) Hearing impairment -	Complex-partial Atyp. absences	Slow activity temporal, occipital	SWMR bilateral (right>left, po, cs, f) HC small bilateral Thalamus left Cortical lesion temporo- parietal right (shunt)	-	No VBM analysis performed	-
EG	BSCP leg dominated (right>left) Severe visual impairment -	Simple partial Complex-partial (neonatal seizures)	*Bilateral, (left>right)* Focal slowing temporo-parietal left	SWMR bilateral (left>right, po, cs, f) HC small left Cerebellum small right	-	No VBM analysis performed	-
TS	Spastic hemiplegia right Leg more affected than arm -	Complex-partial Generalised tonic-clonic (neonatal seizures)	Bilateral isolated sharp waves, predominantly on the left Slow activity generalized bilateral, left>right	SWMR bilateral (left>right, f), gliosis (f) Cerebellum small right	-	No VBM analysis performed	-
PP	BSCP leg dominated (right>left)	Simple partial Complex-partial	Isolated sharp waves fronto-temporal right, Sylvian area left	SWMR bilateral (left>right, po, cs) HC small bilateral	-	No VBM analysis performed	

	Mild dystonic posturing right 76 68 110	Secondary generalisation					
HJ	BSCP three limb dominated (right>left) -	Infantile spasms (neonatal seizures)	Isolated sharp waves frontal, central, parietal, parieto-occipital left Slow activity frontal, fronto-central left	SWMR left (po, cs, f) MCA left Cerebellum small right	-	No VBM analysis performed	-
MC	Suspicious 59 54 68	No epilepsy	No abnormality	MWMR bilateral (po)	0	-	-
RK	Normal 112 96 123	No epilepsy	No abnormality	Normal	0	-	-
DS	Suspicious 123 109 131	No epilepsy	No abnormality	Normal	0	-	-
JoCol	Suspicious 107 99 113	No epilepsy	No abnormality	Normal	0	-	-
AC	Normal 93 85 103	No epilepsy	Sharp transients (theta) temporal left	Normal	0	-	-
VH	Normal 102 118 90	No epilepsy	No abnormality	Normal	0	-	-
CR (I)	Suspicious 88 74 103	No epilepsy	Sharp transients (theta) mixed with sharp waves temporal bilateral, centro-temporal	Normal	0	-	-
JSk	Normal 109 102 113	No epilepsy	No EEG data	Normal	0	-	-
AT	Suspicious 60 54 72	No epilepsy	No abnormality	HC bilateral small	0	-	-
LiWi	Suspicious 103 100 107	No epilepsy	No EEG data	Periventricular gliosis bilateral (right>left, po, f,)	0	-	-
LWa	Normal 100 96 102	No epilepsy	No abnormality	Periventricular gliosis bilateral (po)	0	-	-
NSi	Three limb dominated CP	No epilepsy	No EEG data	Periventricular gliosis bilateral (po, f,)	0	-	-

	Legs more affected than arm 71 57 87						
LH	BSCP leg dominated (right>left) Mild dystonic posturing right 108 96 117	No epilepsy	Isolated sharp waves frontal right	MWMR bilateral (left>right, cs) Periventricular gliosis (cs)	0	-	-
RR (II)	Spastic hemiparesis right Arm and leg equally affected 98 101 96	No epilepsy	Slow activity mixed with *sharp wave complexes* temporal bilateral and right anterior temporal	SWMR (left, cs) Caudate, putamen, thalamus left	0	-	-
GO	Normal 108 99 115	No epilepsy	No abnormality	Periventricular gliosis bilateral (right>left, cs) Right caudate, thalamus left	1-2	Cerebellum left	↓
CS	Suspicious 114 107 117	No epilepsy	Isolated sharp waves anterior temporal right	Normal	1-2	Inferior frontal gyrus left	↑
MD	Normal 105 104 106	No epilepsy	No abnormality	Normal	1-2	Medial orbital gyrus right	↑
ASa	Normal 105 109 102	No epilepsy	No abnormality	Normal	1-2	Thalamus right Thalamus left	↑
SDaW	Suspicious 87 82 93	No epilepsy	No EEG data	Normal	1-2	Superior temporal gyrus right	↓
BA	Normal 96 94 99	No epilepsy	No abnormality	Normal	1-2	Superior/middle temporal gyrus right	↓
SF	Normal 87 88 89	No epilepsy	Isolated sharp waves vertex, fronto-central predominantly left	Normal	1-2	Thalamus left	↑

BBe	Normal 72 58 88	No epilepsy	No abnormality	HC small bilateral	1-2	Superior frontal gyrus right	↓
DSk	Suspicious 109 102 113	No epilepsy	No EEG data	MWMR bilateral (cs) Chiari I	1-2	Cerebellum left	↓
NK	Suspicious 86 75 100	No epilepsy	Slow activity mixed with sharp transients (fronto)-temporal bilateral	Periventricular gliosis (left, po) HC bilateral small Cortical lesion temporal-parietal right (shunt)	1-2	Temporal lobe right (superior temporal gyrus, site of shunt) Cingulate gyrus right	↓
CO (II)	Spastic hemiparesis right Leg more affected than arm 67 56 86	No epilepsy	No abnormality	SWMR bilateral (right>left, right:po,cs, f; left po,f)	>=3	Region of thalamus right Parahippocampal gyrus right Parietal lobe (region of parieto-occipital fissure) right	↓
JRu	Normal 84 76 95	No epilepsy	Sharp components temporal bilateral	Normal	>=3	Thalamus/Gl pallidus/putamen right Thalamus left Angular gyrus left Temporal lobe right (region of superior temporal gyrus ; middle temporal gyrus) Temporal lobe (middle temporal gyrus) left	↑ ↓
DHay	Suspicious 116 109 119	No epilepsy	No definite abnormality (intermitted slow activity temporal bilateral)	MWMR bilateral (po)	>=3	Thalamus right Thalamus left Supramarginal gyrus right Intraparietal sulcus right Frontal lobe (region of middle frontal sulcus) right	↑ ↓

						Precentral gyrus right Parietal lobe (region of intraparietal sulcus left)	
LO(I)	Suspicious Hearing impairment 88 74 103	No epilepsy	Sharp components temporal, frontal bilateral	SWMR right (po,cs), Cortex left parietal (shunt) HC small left Cerebellum small	>=3	Thalamus right Frontal lobe (region of middle frontal gyrus) right Frontal lobe right Caudate left Frontal lobe (region of middle frontal gyrus) left Parietal lobe (region of shunt) left Temporo-parietal right Temporal lobe (region of enlarged ventricle) right Temporo-parietal left Cerebellum left	↑ ↓
CT	Suspicious 54 55 60	No epilepsy	No abnormality	SWMR right, MWMR left (po,cs) HC small bil	-	No VBM analysis performed	-
JC	BSCP leg dominated (right>left) Hearing impairment	No epilepsy	No EEG data	SWMR left, MWMR right (po,cs,f) Cortex left parietal thin (shunt)	-	No VBM analysis performed	-

Absence seizures: typical or atypical absences (see chapter 5 for details); simple absences= only impairment of consciousness, possibly with limited motor activity, e.g. eyelid flattering; complex absences= automatisms or prominent motor components present.

* Visual MRI analysis: W*MR*= white matter reduction (MWMR= mild/moderate; SWMR= severe), BG= basal ganglia, thalamus, MCA= middle cerebral artery, HC=hippocampus; po=parieto-occipital, cs=centrum semiovale, f=frontal

***For MNI co-ordinates of peaks see table 8.2, chapter 8. *** for p=values see table 8.2 chapter 8

$FSIQ= Full Scale Intelligence Quotient, PIQ= Performance Intelligence Quotient, VIQ= Verbal Intelligence Quotient

Appendix 6: Signs of laterality in neurology, seizure type, EEG and voxel-based morphometry analysis

Table A7: Signs of laterality in neurology, seizure type, EEG and VBM grey matter abnormalities in the children with epilepsy

ID	**Neurology (CP) – affected side**	**Focal seizures yes/no Focal clinical signs during seizure**	**EEG abnormality – focal/diffuse slowing**	**EEG abnormality Isolated sharp waves or spikes Sharp wave/spike wave complexes**	**MRI visual assessment – side of abnormalities**	**VBM grey matter abnormality – side of abnormalities**
TM	-	Yes not clear	-	-	-	**Left** insular gyrus
AU	BSCP leg dominated, mild ataxia **(left>right)**	Yes Not clear	Temporal and frontal **bilateral (left>right)**	Frontal **bilateral (left>right)**	WMR **bilateral** Hippocampus **right**	**Right** temporal **Left** frontal Thalamus bilateral
SH	BSCP three limb dominated **(left>right)**	Yes **Right side**	-	**Right** parietal sw complexes, **bilateral** multifocal isolated sw	WMR **bilateral (left>right)**	**Bilateral** cingulate gyrus
LR	-	Yes Not clear	-	**Right** fronto-central **Left** central	WMR **bilateral (right>left)**	**Right** parietal, temporal, hippocampus, putamen **Bilateral** thalamus
TO	-	Yes Not clear	-	**Right** starting then generalised	-	-
SSK	-	Yes Not clear	-	-	-	**Left** cingulate gyrus
EF	Spastic hemiplegia **right**	Yes Not clear	**Bilateral** generalised	**Bilateral** generalised **(left>right)**	WMR **bilateral (left>right)**	**Left** frontal, cerebellum **Right** temporal **Bilateral** parietal
AP	-	Yes	-	**Bilateral**	-	No VBM analysis

		Not clear		generalised		performed
EG	BSCP leg dominated **(right>left)**	Yes **Right side**	**Left** temporo-parietal	**Bilateral (left>right)**	WMR **bilateral (left>right)** Hippocampus **left** (Cerebellum small **right)**	No VBM analysis performed
PP	BSCP leg dominated, mild dystonic posturing right **(right>left)**	Yes Not clear	-	**Right** fronto-temporal **Left** central	WMR **bilateral (left>right)** Hippocampus **bilateral**	No VBM analysis performed
SHay	BSCP leg dominated **(left>right)**	Yes **Left side**	**Bilateral** temporal, occipital	-	WMR **bilateral (right>left)** Hippocampus **bilateral** Thalamus **left** Cortical parietal **right**	No VBM analysis performed
KS	BSCP leg dominated **(left>right)**	Yes Not clear	**Bilateral** fronto-temporal, frontal (**right >left)**	**Bilateral** fronto-temporal, frontal, central	WMR **bilateral (right>left)** BG, Thalamus **bilateral** Hippocampus **right** Cortical frontal, parietal, occipital **right** (Cerebellum small **left)**	No VBM analysis performed
TS	Spastic hemiplegia **right**	Yes Not clear	**Bilateral (left>right)**	**Bilateral (left>right)**	WMR **bilateral (left>right)** Cerebellum small **right**	No VBM analysis performed
PD	BSCP four limb, mild dyskinesia **(left>right)**	No	**Bilateral** frontal	Frontal **bilateral (left>right)**	WMR **bilateral** (Cerebellum small **bilateral)**	**Right** temporal lobe **Bilateral** thalamus, cerebellum
WS	BSCP three limb dominated **(right>left)**	No	-	**Left** mid -temporal	WMR **bilateral (left>right)**	**Right** frontal, parietal, occipital, thalamus **Left** cerebellum
SD	-	No	-	**Bilateral** mid-parietal, central, occipital **(left>right)**	WMR **left** Hippocampus **bilateral**	-
ML	BSCP four limb, ataxia, mild dystonia **(symmetrical)**	No	-	**Left** central-parietal	WMR **bilateral (left>right)** Hippocampus **left** Cortical **left (MCA)** (Cerebellum, pons small)	**Left** temporal, parietal, amygdale/hippocampus **Bilateral** thalamus,cerebellum

TC	-	No	-	**Bilateral** frontal	**WMR bilateral**	**Right** temporal lobe
BK	-	No	**Bilateral** fronto-temporal **(left>right)**	-	Caudate **right**	Superior temporal gyrus **right**
HJ	BSCP three limb dominated **(right>left)**	No	**Left** frontal, fronto-central	**Left** central, parietal, parieto-occipital	WMR **left** Cortical **left (MCA)** (Cerebellum small **right**)	No VBM analysis performed
JW	BSCP leg dominated **(left>right)**	No	**Bilateral** frontal	**Bilateral** frontal (left>right)	WMR **bilateral** Hippocampus **bilateral** Schizencephaly/polymicrogyria **bilateral**	**Bilateral** frontal, temporal, parietal, thalamus **Left** insula, cerebellum
LB	-	No	**Bilateral** temporal/temporo-parietal	-	WMR **bilateral** Hippocampus **bilateral**	Hippocampus **left**
TR	-	No	-	Starting on **right,** generalised	-	No VBM abnormality
AM	Spastic hemiplegia **left**	No	**Bilateral** temporal	**Right** central, frontal	WMR **right** Cortical parietal **right**	**Right** temporo-parietal, parietal, cingulate gyrus

CP = cerebral palsy, BSCP= bilateral spastic cerebral palsy, WMR= periventricular white matter reduction, MCA= middle cerebral artery infarct

Appendix 7: Pre-processing of MRI data for voxel-based mophometry analysis – effect of lesion masks

Table A7: Pre-processing of MRI data for voxel-based morphometry analysis– Results of pre-processing with lesion masks applied

ID	Lesion mask	Normalisation improved	Normalisation successful	Segmentation successful	Excluded from VBM analysis (reason)
LO(II)	Left parietal occipital, right temporal	Yes	Yes	Yes	No
NK	Right temporal parietal	Yes	Yes	Yes	No
CT	Ventricles bilateral	Yes	Yes	Best result without lesion mask but not successful	Yes; segmentation failed
KS	Right hemisphere	No	No	No	Yes; normalisation/segmentation failed
SHay	Right parietal occipital	No	No	No	Yes; normalisation/segmentation failed
ML	Left temporal	Yes	Yes	Borderline	No
EG	Ventricles bilateral	Yes	Yes	No	Yes; segmentation failed
PP	Left ventricle	Yes	Yes	No	Yes; segmentation failed
LR	Right ventricle	Yes	Yes	Yes	No
JW	Bilateral parietal	Yes	Yes	Yes	No
HJ	Left temporal, parietal	No	No	No	Yes